IMMUNOSUPPRESSIVE EFFECTS OF PTERIDINE DERIVATIVES

The invention relates to a class of novel pteridines. The invention further relates to pharmaceutical compositions including a broad class of pteridines especially for the prevention and/or the treatment of pathologic conditions such as, but not limited to, immune and auto-immune disorders, organ and cells transplant rejections, cell proliferative disorders, cardiovascular disorders, disorders of the central nervous system and viral diseases.

The invention further relates to combined pharmaceutical preparations comprising one or more pteridines and one or more known Immuno-suppressant drugs or antineoplastic drugs or anti-viral drugs.

This invention also relates to a method for the prevention and/or treatment of pathologic conditions such as, but not limited to, immune and autoimmune disorders, organ and cells transplant rejections, cell proliferative disorders, cardiovascular disorders, disorders of the central nervous system and viral diseases by the administration of an effective amount of a specific pteridine optionally combined with one or more known immunosuppressant drugs or antineoplastic drugs or anti-viral drugs.

BACKGROUND OF THE INVENTION

Several 2,4-diaminopteridine derivatives being substituted in the 6-position and/or the 7-position of the pteridine ring (according to standard atom numbering for said ring) are known in the art, e.g. from various sources of literature including Swiss Patent No. 231,852; British Patent No. 763,044; United States Patents No. 2,512,572; No. 2,581,889; No. 2,665,275; No. 2,667,486; No. 2,940,972; No. 3,081,230 and No. 5,047,405. Some of these substituted 2,4-diaminopteridine derivatives were disclosed in relationship with various medical uses, such as bacterial growth inhibitors, antineoplastic agents, anti-schisto-somiasis activity, coronary dilating activity, diuretic and hypotensive activity, and anti-amnesic activity. In particular, U.S. Patent No. 2,940,972 and EP-A-362,645 disclose very specific 2,4-diaminopteridine derivatives being substituted by piperidinyl, morpholinyl or pyrrolidinyl in the 7-position of the pteridine ring.

Specific 2-aminopteridine derivatives wherein the 4-position of the pteridine ring is substituted with an alkoxy group and the 6-position is also substituted are also known in the art, although without any medical utility. For instance, United States Patent No. 2,740,784 discloses such derivatives wherein the 6-substituent is an acetal; J. Chem. Soc. (1957) 2146-2158 discloses 2-dimethylamino-4-ethoxy-6-phenylpteridine as a compound with a melting point of 200°C; Arm. Khim. J. discloses 2-amino-4-ethoxy-6,7-diphenylpteridine; Helv. Chem. Acta (1992) 75:2317-2326 discloses 2-amino-4-pentoxy-6-methylthio-pteridine. Furthermore, International Patent Application published as WO 01/19825 discloses 2-phenylamino and 2-phenylsulfoxide pteridines wherein the 7-position is substituted with an amide as being useful inhibitors of cell cycle regulatory kinases.

Nevertheless, there still is a need in the art for specific and highly therapeutically active compounds, such as, but not limited to, drugs for treating immune and autoimmune disorders, organ and cells transplant rejections, cell proliferative disorders, cardiovascular disorders, disorders of the central nervous system and viral diseases. In particular, there is a need in the art to provide immunosuppressive compounds or antineoplastic drugs or anti-viral drugs which are active in a minor dose in order to replace existing drugs having significant side effects and to decrease treatment costs.

Currently used immunosuppressive drugs include antiproliferative agents, such as methotrexate (a 2,4-diaminopteridine derivative disclosed by U.S. Patent No. 2,512,572), azathioprine, and cyclophosphamide. Since these drugs affect mitosis and cell division, they have severe toxic effects on normal cells with high turn-over rate such as bone marrow cells and the gastrointestinal tract lining. Accordingly, marrow depression and liver damage are common side effects of these antiproliferative drugs.

Anti-inflammatory compounds used to induce immunosuppression include adreno-cortical steroids such as dexamethasone and prednisolone. The common side effects observed with the use of these compounds are frequent infections, abnormal metabolism, hypertension, and diabetes.

Other immunosuppressive compounds currently used to inhibit lymphocyte activation and subsequent proliferation include cyclosporine, tacrolimus and rapamycin. Cyclosporine and its relatives are among the most commonly used immunosuppressant drugs. Cyclosporine is typically used for preventing or treating organ rejection in kidney, liver, heart, pancreas, bone marrow, and heart-lung transplants, as well as for the treatment of autoimmune and inflammatory diseases such as Crohn's disease, aplastic anemia, multiple-sclerosis, myasthenia gravis, uveitis, biliary cirrhosis, etc. However, cyclosporines suffer from a small therapeutic dose window and severe toxic effects including nephrotoxicity, hepatotoxicity, hypertension, hirsutism, cancer, and neurotoxicity.

Additionally, monoclonal antibodies with immunosuppressant properties, such as OKT3, have been used to prevent and/or treat graft rejection. Introduction of such monoclonal antibodies into a patient, as with many biological materials, induces several side-effects, such as dyspnea. Within the context of many life-threatening diseases, organ transplantation is considered a standard treatment and, in many cases, the only alternative to death. The immune response to foreign cell surface antigens on the graft, encoded by the major histo-compatibility complex (hereinafter referred as MHC) and present on all cells, generally precludes successful transplantation of tissues and organs unless the transplant tissues come from a compatible donor and the normal immune response is suppressed. Other than identical twins, the best compatibility and thus, long term rates of engraftment, are achieved using MHC identical sibling donors or MHC identical unrelated cadaver donors. However, such ideal matches are difficult to achieve. Further, with the increasing need of donor organs an increasing shortage of transplanted organs currently exists. Accordingly, xenotransplantation

has emerged as an area of intensive study, but faces many hurdles with regard to rejection within the recipient organism.

The host response to an organ allograft involves a complex series of cellular interactions among T and B lymphocytes as well as macrophages or dendritic cells that recognize and are activated by foreign antigen. Co-stimulatory factors, primarily cytokines, and specific cell-cell interactions, provided by activated accessory cells such as macrophages or dendritic cells are essential for T-cell proliferation. These macrophages and dendritic cells either directly adhere to T-cells through specific adhesion proteins or secrete cytokines that stimulate T-cells, such as IL-12 and IL-15. Accessory cell-derived co-stimulatory signals stimulate activation of interleukin-2 (IL-2) gene transcription and expression of high affinity IL-2 receptors in T-cells. IL-2 is secreted by T lymphocytes upon antigen stimulation and is required for normal immune responsiveness. IL-2 stimulates lymphoid cells to proliferate and differentiate by binding to IL-2 specific cell surface receptors (IL-2R). IL-2 also initiates helper T-cell activation of cytotoxic T-cells and stimulates secretion of interferon-γ which in turn activates cytodestructive properties of macrophages. Furthermore, IFN-γ and IL-4 are also important activators of MHC class II expression in the transplanted organ, thereby further expanding the rejection cascade by enhancing the immunogenicity of the grafted organ The current model of a T-cell mediated response suggests that T-cells are primed in the T-cell zone of secondary lymphoid organs, primarily by dendritic cells. The initial interaction requires cell to cell contact between antigen-loaded MHC molecules on antigen-presenting cells (hereinafter referred as APC) and the T-cell receptor/CD3 complex on T-cells. Engagement of the TCR/CD3 complex induces CD154 expression predominantly on CD4 T-cells that in turn activate the APC through CD40 engagement, leading to improved antigen presentation. This is caused partly by upregulation of CD80 and CD86 expression on the APC, both of which are ligands for the important CD28 co-stimulatory molecule on T-cells. However, engagement of CD40 also leads to prolonged surface expression of MHC-antigen complexes, expression of ligands for 4-1BB and OX-40 (potent co-stimulatory molecules expressed on activated T-cells). Furthermore, CD40 engagement leads to secretion of various cytokines (e.g., IL-12, IL-15, TNF-α, IL-1, IL-6, and IL-8) and chemokines, all of which have important effects on both APC and T-cell activation and maturation. Similar mechanisms are involved in the development of auto-immune disease, such as type I diabetes. In humans and non-obese diabetic mice, insulin-dependent diabetes mellitus results from a spontaneous T-cell dependent auto-immune destruction of insulin-producing pancreatic .beta. cells that intensifies with age. The process is preceded by infiltration of the islets with mononuclear cells (insulitis), primarily composed of T lymphocytes. A delicate balance between auto-aggressive T-cells and suppressor-type immune phenomena determines whether expression of auto-immunity is limited to insulitis or not. Therapeutic strategies that target T-cells have been successful in preventing further progress of the auto-immune disease. These include neonatal thymectomy, administration of cyclosporine, and infusion of anti-pan T-cell, anti-CD4, or anti-CD25 (IL-2R) monoclonal antibodies. The aim of all rejection prevention and auto-immunity reversal

strategies is to suppress the patient's immune reactivity to the antigenic tissue or agent, with a minimum of morbidity and mortality. Accordingly, a number of drugs are currently being used or investigated for their immunosuppressive properties. As discussed above, the most commonly used immunosuppressant is cyclosporine, which however has numerous side effects. Accordingly, in view of the relatively few choices for agents effective at immunosuppression with low toxicity profiles and manageable side effects, there exists a need in the art for identification of alternative immunosuppressive agents and for agents acting as complement to calcineurin inhibition.

The metastasis of cancer cells represents the primary source of clinical morbidity and mortality in the large majority of solid tumors. Metastasis of cancer cells may result from the entry of tumor cells into either lymphatic or blood vessels. Invasion of lymphatic vessels results in metastasis to regional draining lymph nodes. From the lymph nodes, melanoma cells for example tend to metastasize to the lung, liver, and brain. For several solid tumors, including melanoma, the absence or the presence of lymph nodes metastasis is the best predictor of patient survival. Presently, to our knowledge, no treatment is capable of preventing or significantly reducing metastasis. Hence, there is a need in the art for compounds having such anti-metastasis effect for a suitable treatment of cancer patients.

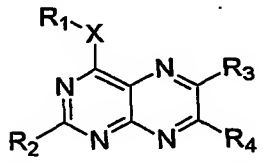
In the field of allergy, IgE is well known for inducing allergy mainly by stimulating mast cells to release histamine. Also, asthma, being characterized by inflammation of airway and bronchospasm, is mainly induced by Th2 cytokines such as IL-5, IL-10 or IL-13. Therefore there is a need in the art for compounds that efficiently inhibit the release of these Th2 cytokines.

There is also a need in the art to improve therapeutic efficiency by providing pharmaceutical compositions or combined preparations exhibiting a synergistic effect as a result of combining two or more immunosuppressant drugs, or antineoplastic drugs or anti-viral drugs or anti-histamine drugs.

Meeting these various needs in the art constitutes the main goal of the present invention.

SUMMARY OF THE INVENTION

In a first embodiment, the present invention relates to a group of novel pteridine derivatives having the general formula (I):



wherein X represents an oxygen atom or a group with the formula $S(O)_m$ wherein m is an integer from 0 to 2, or a group with the formula NZ and wherein:

- R_1 is a group selected from the group consisting of C_{1-7} alkyl, C_{2-7} alkenyl, C_{2-7} alkynyl, C_{3-10} cycloalkyl, C_{3-10} cycloalkenyl, aryl, alkylaryl, arylalkyl, heterocyclic, heterocyclic-substituted alkyl and alkyl-substituted heterocyclic, each of said groups being optionally substituted with one or more substituents selected from the group consisting of halogen, C_{1-4} alkyl, C_{1-4} alkoxy, C_{2-7} alkenyl, C_{2-7} alkynyl, halo C_{1-4} alkyl, C_{3-10} cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, hydroxyl, sulfhydryl, nitro, hydroxylamino, mercaptoamino, cyano, carboxylic acid or esters or thioesters or amides or thioamides or halides or anhydrides thereof, thiocarboxylic acid or esters or thioesters or amides or thioamides or halides or anhydrides thereof, carbamoyl, thiocarbamoyl, ureido, thio-ureido, amino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkyl-amino, hydroxylalkylamino, mercaptoalkyl-amino, heterocyclic amino, hydrazino, alkylhydrazino and phenyl-hydrazino; or R_1 is a carboxyalkyl, carboxyaryl, thiocarboxyaryl or thiocarboxyalkyl group;
- Z is a group independently defined as R_1 or Z is hydrogen or the group NZ together with R_1 is either hydroxylamino or an optionally substituted heterocyclic group containing at least one nitrogen atom;
- R_2 is selected from the group consisting of amino; acylamino; thioacylamino; carbamoyl; thiocarbamoyl, ureido; thio-ureido, sulfonamido; hydroxylamino; alkoxyamino; thioalkylamino; mercaptoamino, hydrazino; alkylhydrazino; phenylhydrazino; optionally substituted heterocyclic radicals; C_{3-7} alkylamino; arylamino; arylalkyl-amino; cycloalkylamino; alkenylamino; cycloalkenylamino; heterocyclic amino; hydroxyalkylamino; mercaptoalkylamino; C_{1-7} alkoxy; C_{3-10} cycloalkoxy; thio C_{1-7} alkyl; arylsulfoxide; arylsulfone; heterocyclic sulfoxide; heterocyclic sulfone; thio C_{3-10} cycloalkyl; aryloxy; arylthio; arylalkyloxy; arylalkylthio; oxyheterocyclic and thioheterocyclic radicals;
- R_4 is an atom or a group selected from the group consisting of hydrogen; halogen; C_{1-7} alkyl; C_{2-7} alkenyl; C_{2-7} alkynyl; halo C_{1-7} alkyl; carboxy C_{1-7} alkyl; carboxyaryl; C_{1-7} alkoxy; C_{3-10} cycloalkoxy; aryloxy; arylalkyloxy; oxyheterocyclic; heterocyclic-substituted alkyloxy; thio C_{1-7} alkyl; thio C_{3-10} cycloalkyl; thioaryl; thioheterocyclic; arylalkylthio; heterocyclic-substituted alkylthio; hydroxylamino; mercapto-amino; acylamino; thio-acylamino; alkoxyamino; thioalkylamino; acetal; thio-acetal; carboxylic acid; carboxylic acid esters, thioesters, halides, anhydrides, amides and thioamides; thiocarboxylic acid; thiocarboxylic acid esters, thioesters, halides, anhydrides, amides and thioamides; hydroxyl; sulfhydryl; nitro; cyano; carbamoyl; thiocarbamoyl, ureido; thio-ureido; alkylamino; cycloalkylamino; alkenylamino; cycloalkenylamino; alkynylamino; arylamino; arylalkylamino; hydroxyalkylamino; mercaptoalkylamino; heterocyclic amino; heterocyclic-substituted alkylamino; oximino; alkyloximino; hydrazino; alkylhydrazino; phenylhydrazino; cysteinyl acid, esters, thioesters, halides, anhy-

drides, amides and thioamides thereof; phenyl substituted with one or more substituents selected from the group consisting of C_{1-7} alkyl, C_{2-7} alkenyl, C_{2-7} alkynyl, halo C_{1-7} alkyl, nitro, hydroxy, sulfhydryl, amino, C_{3-10} cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, carbamoyl, thiocarbamoyl, ureido, thio-ureido, sulfonamido, hydroxylamino, alkoxyamino, mercaptoamino, thioalkylamino, acylamino, thioacylamino, cyano, carboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, thiocarboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino and phenylhydrazino; aryl groups other than phenyl, the said aryl groups being optionally substituted with one or more substituents selected from the group consisting of halogen, C_{1-7} alkyl, C_{1-7} alkoxy, C_{2-7} alkenyl, C_{2-7} alkynyl, halo C_{1-7} alkyl, nitro, hydroxyl, sulfhydryl, amino, C_{3-10} cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, carbamoyl, thiocarbamoyl, ureido, thio-ureido, sulfonamido, hydroxylamino, alkoxyamino, mercaptoamino, thioalkylamino, acylamino, thioacylamino, cyano, carboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, thiocarboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino and phenylhydrazino; optionally substituted heterocyclic radicals other than piperidinyl, morpholinyl or pyrrolidinyl, i.e. preferably selected from the group consisting of oxabicycloheptyl, azabenzimidazolyl, azacycloheptyl, azacyclooctyl, azacyclononyl, azabicyclononyl, tetrahydrofuryl, tetrahydropyranyl, tetrahydropyronyl, tetrahydroquinoleinyl, tetrahydrothienyl and dioxide thereof, dihydrothienyl dioxide, dioxindolyl, dioxinyl, dioxenyl, dioxazinyl, thioxanyl, thioxolyl, thio-urazolyl, thiotriazolyl, thiopyranyl, thiopyronyl, coumarinyl, quinoleinyl, oxyquinoleinyl, quinuclidinyl, xanthinyl, dihydropyranyl, benzodihydrofuryl, benzothiopyronyl, benzothiopyranyl, benzoxazinyl, benzoxazolyl, benzodioxolyl, benzodioxanyl, benzothiadiazolyl, benzotriazinyl, benzothiazolyl, benzoxazolyl, phenothioxinyl, phenothiazolyl, phenothienyl, phenopyronyl, phenoxazolyl, pyridinyl, dihydropyridinyl, tetrahydro-pyridinyl, piperidinyl, thiomorpholinyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, tetrazinyl, triazolyl, benzotriazolyl, tetrazolyl, imidazolyl, pyrazolyl, thiazolyl, thiadiazolyl, isothiazolyl, oxazolyl, oxadiazolyl, pyrrolyl, furyl, dihydrofuryl, furoyl, hydantoinyl, dioxolanyl, dioxolyl, dithianyl, dithienyl, dithiinyl, thienyl, indolyl, indazolyl, benzofuryl, quinolyl, quinazolinyl, quinoxalinyl, carbazolyl, phenoxazinyl, phenothiazinyl, xanthenyl, purinyl, benzothienyl, naphtothienyl, thianthrenyl, pyranyl, pyronyl, benzopyronyl, isobenzo-

furanyl, chromenyl, phenoxathiinyl, indolizinyl, quinolizinyl, isoquinolyl, phthalazinyl, naphthiridinyl, cinnolinyl, pteridinyl, carbolinyl, acridinyl, perimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, imidazolinyl, imidazolidinyl, benzimidazolyl, pyrazolinyl, pyrazolidinyl, pyrrolinyl, pyrrolidinyl, piperazinyl, uridinyl, thymidinyl,cytidinyl, aziridinyl, aziridinyl, diaziridinyl, diaziridinyl, oxiranyl, oxaziridinyl, dioxiranyl, thiiranyl, azetyl, dihydroazetyl, azetidinyl, oxetyl, oxetanyl, thietyl, thietanyl, diazabicyclooctyl, diazetyl, diaziridinonyl, diaziridinethionyl, chromanyl, chromanonyl, thiochromanyl, thiochromanonyl, thiochromenyl, benzofuranyl, benzisothiazolyl, benzocarbazolyl, benzochromonyl, benzisoalloxazinyl, benzocoumarinyl, thiocoumarinyl, phenometoxazinyl, phenoparoxazinyl, phentriazinyl, thiodiazinyl, thiodiazolyl, indoxyl, thioindoxyl, benzodiazinyl, phtalidyl, phtalimidinyl, phtalazonyl, alloxazinyl, xanthionyl, isatyl, isopyrazolyl, isopyrazolonyl, urazolyl, urazinyl, uretinyl, uretidinyl, succinyl, succinimido, benzylsultimyl and benzylsultamyl; aromatic or heterocyclic substituents substituted with an aliphatic spacer between the pteridine ring and the aromatic or heterocyclic substituent, whereby said aliphatic spacer is a branched or straight, saturated or unsaturated aliphatic chain of 1 to 4 carbon atoms which may contain one or more functions, atoms or radicals selected from the group consisting of carbonyl (oxo), thiocarbonyl, alcohol (hydroxyl), thiol, ether, thioether, acetal, thioacetal, amino, imino, oximino, alkyloximino, amino-acid, cyano, acylamino, thioacylamino, carbamoyl, thiocarbamoyl, ureido, thioureido, carboxylic acid or ester or thioester or halide or anhydride or amide, thiocarboxylic acid or ester or thioester or halide or anhydride or amide, nitro, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, hydroxylamino, mercaptoamino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and halogen; branched or straight, saturated or unsaturated aliphatic chains of 1 to 7 carbon atoms optionally containing one or more functions selected from the group consisting of carbonyl (oxo), thiocarbonyl, alcohol (hydroxyl), thiol, ether, thioether, acetal, thioacetal, amino, imino, oximino, alkyloximino, aminoacid, cyano, acylamino; thioacylamino; carbamoyl, thiocarbamoyl, ureido, thioureido, carboxylic acid ester or halide or anhydride or amide, thiocarboxylic acid or ester or thioester or halide or anhydride or amide, nitro, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, hydroxylamino, mercaptoamino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and halogen; and

- R_3 is an atom or a group selected from the group consisting of fluoro, bromo, iodo, C_{2-7} alkyl; C_{2-7} alkenyl; C_{2-7} alkynyl; halo C_{1-7} alkyl; C_{1-7} alkoxy; C_{3-10} cycloalkoxy; aryloxy; arylalkyloxy; oxyheterocyclic; heterocyclic-substituted alkyloxy; thio C_{2-7} alkyl; thio C_{3-10} cycloalkyl; thioaryl; thioheterocyclic; arylalkylthio; heterocyclic-substituted alkylthio; hydroxylamino; alkoxyamino; thioalkylamino; mercaptoamino; acyl-amino;

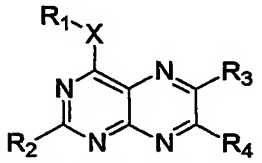
thio-acylamino; thio-acetal; carboxylic acid; carboxylic acid esters, thioesters, amides, halides, anhydrides and thioamides; thiocarboxylic acid; thiocarboxylic acid esters, thioesters, amides, halides, anhydrides and thioamides; hydroxyl; sulfhydryl; nitro; carbamoyl; thiocarbamoyl; ureido; thio-ureido; amino; alkylamino; cycloalkylamino; alkenylamino; cycloalkenylamino; alkynylamino; arylamino; arylalkylamino; hydroxyalkylamino; mercaptoalkylamino; heterocyclic amino; heterocyclic-substituted alkylamino; oximino; alkyloximino; hydrazino; alkylhydrazino; phenylhydrazino; cysteinyl acid, esters, thioesters, amides and thioamides thereof; aryl optionally substituted with one or more substituents selected from the group consisting of halogen, C_{1-7} alkyl, C_{1-7} alkoxy, C_{2-7} alkenyl, C_{2-7} alkynyl, halo C_{1-7} alkyl, nitro, hydroxyl, sulfhydryl, amino, C_{3-10} cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, carbamoyl, thiocarbamoyl, ureido, thio-ureido, sulfonamido, hydroxylamino, mercaptoamino, alkoxyamino, thioalkylamino, acylamino, thioacylamino, cyano, carboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, thiocarboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxylalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino and phenylhydrazino; optionally substituted heterocyclic radicals; aromatic or heterocyclic substituents substituted with an aliphatic spacer between the pteridine ring and the aromatic or heterocyclic substituent, whereby said aliphatic spacer is a branched or straight, saturated or unsaturated aliphatic chain of 1 to 4 carbon atoms which may contain one or more functions, atoms or radicals selected from the group consisting of carbonyl (oxo), thiocarbonyl, alcohol (hydroxyl), thiol, ether, thio-ether, acetal, thio-acetal, amino, imino, oximino, alkyloximino, amino-acid, cyano, carboxylic acid or ester or thioester or amide, nitro, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkyl-amino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfonamido and halogen; branched or straight, saturated or unsaturated aliphatic chains of 2 to 7 carbon atoms optionally containing one or more functions selected from the group consisting of thiocarbonyl, alcohol (hydroxyl), thiol, ether, thioether, thioacetal, amino, imino, oximino, alkyloximino, amino-acid, cyano, acylamino, thioacylamino, carbamoyl, thiocarbamoyl, ureido, thioureido, carboxylic acid or ester or thioester or halide or anhydride or amide, thio carboxylic acid or ester or thioester or halide or anhydride or amide, nitro, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, hydroxylamino, mercaptoamino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and

halogen; or R_3 together with R_4 forms a homocyclic or heterocyclic radical such as, but not limited to, indolyl, dihydroxypyrimidyl or tetramethylene;

and/or a pharmaceutically acceptable addition salt thereof and/or a stereoisomer thereof and/or a mono- or a di-*N*-oxide thereof and/or a solvate thereof and/or a dihydro- or tetrahydropteridine derivative thereof.

The above novel compounds have in common the structural features present in the general formula (I), in particular they are at least trisubstituted in positions 2, 4 and 6 of the pteridine ring. They also have a potential specific biological activity profile and consequent usefulness in medicinal chemistry.

In a second embodiment, the present invention relates to the unexpected finding that at least one desirable biological property such as, but not limited to, the ability to decrease the proliferation of lymphocytes, or to decrease T-cell activation, or to decrease B-cell or monocytes or macrophages activation, or to inhibit the release of certain cytokines, is a common feature which is not only present in the group of novel compounds defined in the general formula (I), but also in a group of pteridine derivatives which is broader than the said group of novel compounds. As a consequence, the invention relates to pharmaceutical compositions comprising as an active principle at least one pteridine derivative having the general formula (II):



wherein X represents an oxygen atom or a group with the formula $S(O)_m$ wherein m is an integer from 0 to 2, or a group with the formula NZ and wherein:

- R_1 is a group selected from the group consisting of C_{1-7} alkyl, C_{2-7} alkenyl, C_{2-7} alkynyl, C_{3-10} cycloalkyl, C_{3-10} cycloalkenyl, aryl, alkylaryl, arylalkyl, heterocyclic, heterocyclic-substituted alkyl and alkyl-substituted heterocyclic, each of said groups being optionally substituted with one or more substituents selected from the group consisting of halogen, C_{1-4} alkyl, C_{1-4} alkoxy, C_{2-7} alkenyl, C_{2-7} alkynyl, halo C_{1-4} alkyl, C_{3-10} cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, hydroxyl, sulfhydryl, nitro, hydroxylamino, mercaptoamino, cyano, carboxylic acid or esters or thioesters or amides or thioamides or halides or anhydrides thereof, thiocarboxylic acid or esters or thioesters or amides or thioamides or halides or anhydrides thereof, carbamoyl, thiocarbamoyl, ureido, thio-ureido, amino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkyl-amino, hydroxylalkylamino, mercaptoalkyl-amino, heterocyclic amino, hydrazino, alkylhydrazino and phenyl-hydrazino; or R_1 is a carboxyalkyl, carboxyaryl, thiocarboxyaryl or thiocarboxyalkyl group;

- Z is a group independently defined as R_1 or Z is hydrogen or the group NZ together with R_1 is either hydroxylamino or an optionally substituted heterocyclic group containing at least one nitrogen atom;
- R_2 is selected from the group consisting of amino; acylamino; thioacylamino; carbamoyl; thiocarbamoyl, ureido; thioureido, sulfon-amido; hydroxylamino; alkoxyamino; thioalkylamino; mercaptoamino, hydrazino; alkylhydrazino; phenylhydrazino; optionally substituted heterocyclic radicals; C_{3-7} alkylamino; arylamino; arylalkylamino; cycloalkylamino; alkenylamino; cycloalkenylamino; heterocyclic amino; hydroxyalkylamino; mercaptoalkylamino; C_{1-7} alkoxy; C_{3-10} cycloalkoxy; thio C_{1-7} alkyl; arylsulfoxide; arylsulfone; heterocyclic sulfoxide; heterocyclic sulfone; thio C_{3-10} cycloalkyl; aryloxy; arylthio; arylalkyloxy; arylalkylthio; oxyheterocyclic and thioheterocyclic radicals,
- R_4 is an atom or a group selected from the group consisting of hydrogen; halogen; C_{1-7} alkyl; C_{2-7} alkenyl; C_{2-7} alkynyl; halo C_{1-7} alkyl; carboxy C_{1-7} alkyl; acetoxy C_{1-7} alkyl; carboxyaryl; C_{1-7} alkoxy; C_{3-10} cycloalkoxy; aryloxy; arylalkyloxy; oxyheterocyclic; heterocyclic-substituted alkyloxy; thio C_{1-7} alkyl; thio C_{3-10} cycloalkyl; thioaryl; thioheterocyclic; arylalkylthio; heterocyclic-substituted alkylthio; hydroxylamino; mercapto-amino; acylamino; thio-acylamino; alkoxyamino; thioalkylamino; acetal; thio-acetal; carboxylic acid; carboxylic acid esters, thioesters, halides, anhydrides, amides and thioamides; thiocarboxylic acid; thiocarboxylic acid esters, thioesters, halides, anhydrides, amides and thioamides; hydroxyl; sulfhydryl; nitro; cyano; carbamoyl; thiocarbamoyl, ureido; thio-ureido; alkylamino; cycloalkylamino; alkenylamino; cycloalkenylamino; alkynylamino; arylamino; arylalkylamino; hydroxyalkylamino; mercaptoalkylamino; heterocyclic amino; heterocyclic-substituted alkylamino; oximino; alkyloximino; hydrazino; alkylhydrazino; phenylhydrazino; cysteinyl acid, esters, thioesters, halides, anhydrides, amides and thioamides thereof; phenyl substituted with one or more substituents selected from the group consisting of C_{1-7} alkyl, C_{2-7} alkenyl, C_{2-7} alkynyl, halo C_{1-7} alkyl, nitro, hydroxyl, sulfhydryl, amino, C_{3-10} cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, carbamoyl, thiocarbamoyl, ureido, thio-ureido, sulfonamido, hydroxylamino, alkoxyamino, mercaptoamino, thioalkylamino, acylamino, thioacylamino, cyano, carboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, thiocarboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, alkylamino, cycloalkylamino, alkenyl-amino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino and phenylhydrazino; aryl groups other than phenyl, the said aryl groups being optionally substituted with one or more substituents selected from the group consisting of halogen, C_{1-7} alkyl, C_{1-7} alkoxy, C_{2-7} alkenyl, C_{2-7} alkynyl, halo C_{1-7} alkyl, nitro,

hydroxyl, sulfhydryl, amino, C_{3-10} cycloalkoxy, aryloxy, arylalkyloxy, oxyhetero-cyclic, heterocyclic-substituted alkyloxy, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, carbamoyl, thiocarbamoyl, ureido, thio-ureido, sulfonamido, hydroxylamino, alkoxyamino, mercaptoamino, thioalkylamino, acylamino, thioacylamino, cyano, carboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, thiocarboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, alkylamino, cycloalkylamino, alkenylamino, cyclo-alkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkyl-amino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkyl-hydrazino and phenylhydrazino; optionally substituted heterocyclic radicals other than piperidinyl, morpholinyl or pyrrolidinyl, i.e. preferably selected from the group consisting of oxabicycloheptyl, azabenzimi-dazolyl, azacycloheptyl, azacyclooctyl, azacyclononyl, azabicyclononyl, tetrahydrofuryl, tetrahydropyranyl, tetrahydropyronyl, tetrahydroquino-leinyl, tetrahydrothienyl and dioxide thereof, dihydrothienyl dioxide, dioxindolyl, dioxinyl, dioxenyl, dioxazinyl, thioxanyl, thioxolyl, thio-urazolyl, thiotriazolyl, thiopyranyl, thiopyronyl, coumarinyl, quinoleinyl, oxyquinoleinyl, quinuclidinyl, xanthinyl, dihydropyranyl, benzodihydro-furyl, benzothiopyronyl, benzothiopyranyl, benzoxazinyl, benzoxazolyl, benzodioxolyl, benzodioxanyl, benzothiadiazolyl, benzotriazinyl, benzothiazolyl, benzoxazolyl, phenothioxinyl, phenothiazolyl, phenothienyl, phenopyronyl, phenoxazolyl, pyridinyl, dihydropyridinyl, tetrahydropyridinyl, piperidinyl, thiomorpholinyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, tetrazinyl, triazolyl, benzotriazolyl, tetrazolyl, imidazolyl, pyrazolyl, thiazolyl, thiadiazolyl, isothiazolyl, oxazolyl, oxadiazolyl, pyrrolyl, furyl, dihydrofuryl, furoyl, hydantoinyl, dioxolanyl, dioxolyl, dithianyl, dithienyl, dithiinyl, thienyl, indolyl, indazolyl, benzofuryl, quinolyl, quinazolinyl, quinoxalinyl, carbazolyl, phenoxazinyl, phenothiazinyl, xanthenyl, purinyl, benzothienyl, naphtothienyl, thianthrenyl, pyranyl, pyronyl, benzopyronyl, isobenzo-furanyl, chromenyl, phenoxathiinyl, indolizinyl, quinolizinyl, isoquinolyl, phthalazinyl, naphthiridinyl, cinnolinyl, pteridinyl, carbolinyl, acridinyl, perimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, imidazolinyl, imidazolidinyl, benzimidazolyl, pyrazolinyl, pyrazolidinyl, pyrrolinyl, pyrrolidinyl, piperazinyl, uridinyl, thymidinyl, cytidinyl, azirinyl, aziridinyl, diazirinyl, diaziridinyl, oxiranyl, oxaziridinyl, dioxiranyl, thiiranyl, azetyl, dihydroazetyl, azetidinyl, oxetyl, oxetanyl, thietyl, thietanyl, diaza-bicyclooctyl, diazetyl, diaziridinonyl, diaziridinethionyl, chromanyl, chromanonyl, thiochromanyl, thiochromanonyl, thiochromenyl, benzofuranyl, benzisothiazolyl, benzocarbazolyl, benzochromonyl, benziso-alloxazinyl, benzocoumarinyl, thiocoumarinyl, phenometoxa-zinyl, phenoparoxazinyl, phentriazinyl, thiodiazinyl, thiodiazolyl, indoxyl, thioindoxyl, benzodiazinyl, phtalidyl, phtalimidinyl, phtalazonyl, alloxazinyl, xanthionyl, isatyl, isopyrazolyl, isopyrazolonyl, urazolyl, urazinyl, uretinyl, uretidinyl, succinyl, succinimido, benzylsultimyl and benzylsultamyl; aromatic or heterocyclic substituents

substituted with an aliphatic spacer between the pteridine ring and the aromatic or heterocyclic substituent, whereby said aliphatic spacer is a branched or straight, saturated or unsaturated aliphatic chain of 1 to 4 carbon atoms which may contain one or more functions, atoms or radicals selected from the group consisting of carbonyl (oxo), thiocarbonyl, alcohol (hydroxyl), thiol, ether, thio-ether, acetal, thio-acetal, amino, imino, oximino, alkyloximino, amino-acid, cyano, acylamino, thioacylamino, carbamoyl, thiocarbamoyl, ureido, thio-ureido, carboxylic acid or ester or thioester or halide or anhydride or amide, thiocarboxylic acid or ester or thioester or halide or anhydride or amide, nitro, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, hydroxylamino, mercaptoamino, alkylamino, cycloalkyl-amino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and halogen; branched or straight, saturated or unsaturated aliphatic chains of 2 to 7 carbon atoms optionally containing one or more functions selected from the group consisting of carbonyl (oxo), thiocarbonyl, alcohol (hydroxyl), thiol, ether, thio-ether, acetal, thio-acetal, amino, imino, oximino, alkyl-oximino, amino-acid, cyano, acylamino; thioacylamino; carbamoyl, thiocarbamoyl, ureido, thio-ureido, carboxylic acid ester or halide or anhydride or amide, thiocarboxylic acid or ester or thioester or halide or anhydride or amide, nitro, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, hydroxylamino, mercapto-amino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxy-alkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and halogen; and

- R_3 is an atom or a group defined as R_4 or R_3 is selected from the group consisting of morpholinyl, amino, hydrogen, methyl, thiomethyl and chloro; or R_3 together with R_4 forms a homocyclic or heterocyclic radical such as, but not limited to, indolyl, dihydroxypyrimidyl or tetramethylene;

and/or a pharmaceutically acceptable addition salt thereof and/or a stereoisomer thereof and/or a mono- or a di-*N*-oxide thereof and/or a solvate and/or a dihydro- or tetrahydropteridine derivative thereof.

Compounds of formula (II) are highly active immunosuppressive agents, antineoplastic agents, anti-allergic agents or anti-viral agents which, together with one or more pharmaceutically acceptable carriers, may be formulated into pharmaceutical compositions for the prevention or treatment of pathologic conditions such as, but not limited to, immune and autoimmune disorders, organ and cells transplant rejections, allergic conditions, cell proliferative disorders, cardiovascular disorders, disorders of the central nervous system and viral diseases.

In a further embodiment, the present invention relates to combined preparations containing at least one compound of formula (II) and one or more drugs such as immunosuppressant and/or immunomodulator drugs, antineoplastic drugs, anti-histamines, inhibitors of agents

causative of allergic conditions, or antiviral agents. In a further embodiment, the present invention relates to the prevention or treatment of the above-cited pathologic conditions by administering to the patient in need thereof an effective amount of a compound of general formula (II), optionally in the form of a pharmaceutical composition or combined preparation with another suitable drug.

In a still further embodiment, the present invention relates to various processes and methods for making the novel pteridine derivatives defined in general formula (I), as well as their pharmaceutically acceptable salts, N-oxides, solvates, enantiomers and dihydro- and tetrahydroderivatives.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents a scheme for the preparation of 2,4,6-trisubstituted pteridine derivatives according to one embodiment of this invention, having various R_2 and R_3 substituents in the 2- and 6- positions of the pteridine ring, respectively.

Figures 2 to 5 represent alternative schemes for the preparation of 2,4,6- or 2,4,7-trisubstituted or 2,4,6,7-tetrasubstituted pteridine derivatives with various R_1, R_2, R_3 and/or R_4 substituents, according to other embodiments of this invention.

Figure 6 represents a scheme for the preparation of symmetrical 2,4,6- trisubstituted pteridines and 2,4,7-trisubstituted as well as 2,4,6,7-tetrasubstituted pteridine derivatives according to another embodiment of this invention, i.e. wherein substituents in the 2- and 4-positions of the pteridine ring are identical.

Figure 7 represents a scheme for the preparation of 2,4,6-trisubstituted pteridine derivatives according to one embodiment of this invention, wherein the substituent in the 6-position of the pteridine ring is a phenyl group substituted by a nitrogen-containing function.

Figure 8 represents a scheme for the preparation of 2,4,6-trisubstituted pteridine derivatives according to another embodiment of this invention, wherein the substituent in the 6- position of the pteridine ring is a phenyl group substituted by an oxygen-containing function.

DEFINITIONS

Unless otherwise stated herein, the term " trisubstituted " means that three of the carbon atoms being in positions 2, 4 and 6 or, alternatively, in positions 2, 4 and 7 of the pteridine ring (according to standard atom numbering for the pteridine ring) are substituted with an atom or group other than hydrogen. The term " tetrasubstituted " means that all four carbon atoms being in positions 2, 4, 6 and 7 of the pteridine ring are substituted with an atom or group other than hydrogen.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms " C_{1-7} alkyl" or " aliphatic saturated hydrocarbon radicals with 1 to 7 carbon atoms "

means straight and branched chain saturated acyclic hydrocarbon monovalent radicals having from 1 to 7 carbon atoms such as, for example, methyl, ethyl, propyl, n-butyl, 1-methylethyl (isopropyl), 2-methylpropyl (isobutyl), 1,1-dimethylethyl (ter-butyl), 2-methylbutyl, n-pentyl, dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, n-heptyl and the like; the term " C_{1-4} alkyl " designate the corresponding radicals with only 1 to 4 carbon atoms, and so on.

As used herein with respect to a substituting radical, and unless otherwise stated, the term C_{1-7} alkylene means the divalent hydrocarbon radical corresponding to the above defined C_{1-7} alkyl, such as methylene, bis(methylene), tris(methylene), tetramethylene, hexamethylene and the like.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms " C_{3-10} cycloalkyl" and " cycloaliphatic saturated hydrocarbon radical with 3 to 10 carbon atoms " means a monocyclic saturated hydrocarbon monovalent radical having from 3 to 10 carbon atoms, such as for instance cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and the like, or a C_{7-10} polycyclic saturated hydrocarbon monovalent radical having from 7 to 10 carbon atoms such as, for instance, norbornyl, fenchyl, trimethyltricycloheptyl or adamantyl.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " C_{3-10} cycloalkylene" means the divalent hydrocarbon radical corresponding to the above defined C_{3-10} cycloalkyl.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms " aryl " and "aromatic substituent " are interchangeable and designate any mono- or polyaromatic monovalent hydrocarbon radical having from 6 up to 30 carbon atoms such as but not limited to phenyl, naphthyl, anthracenyl, adamantyl, phenantracyl, fluoranthenyl, chrysenyl, pyrenyl, biphenylyl, terphenyl, picenyl and the like, including spiro hydrocarbon radicals and fused benzo - C_{5-8} cycloalkyl radicals (the latter being as defined above) such as, for instance, indanyl, 1,2,3,4-tetrahydronaphtalenyl, fluorenyl and the like.

As used herein with respect to a substituting radical such as the combination of R_3 and R_4, and unless otherwise stated, the term " homo-cyclic" means a mono- or polycyclic, saturated or mono-unsaturated or polyunsaturated hydrocarbon radical having from 4 up to 15 carbon atoms but including no heteroatom in the said ring.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " heterocyclic " means a mono- or polycyclic, saturated or mono-unsaturated or polyunsaturated monovalent hydrocarbon radical having from 2 up to 15 carbon atoms and including one or more heteroatoms in a 3 to 10 membered ring (and optionally one or more heteroatoms attached to one or more carbon atoms of said ring, for instance in the form of a carbonyl or thiocarbonyl group) and/or to one or more heteroatoms of said ring, for instance in the form of a sulfone, sulfoxide, N-oxide, phosphate, phosphonate or selenium oxide, each said heteroatom being independently selected from the group consisting of nitrogen, oxygen, sulfur, selenium and phosphorus, including benzo-fused heterocyclic radicals, such as but not limited to oxabicycloheptyl, azabenzimidazolyl, azacycloheptyl, azacyclooctyl, azacyclononyl,

azabicyclononyl, tetrahydrofuryl, tetrahydropyranyl, tetrahydropyronyl, tetrahydroquinoleinyl, tetrahydrothienyl and dioxide thereof, dihydrothienyl dioxide, dioxindolyl, dioxinyl, dioxenyl, dioxazinyl, thioxanyl, thioxolyl, thio-urazolyl, thiotriazolyl, thiopyranyl, thiopyronyl, coumarinyl, quinoleinyl, oxyquinoleinyl, quinuclidinyl, xanthinyl, dihydropyranyl, benzodihydrofuryl, benzothiopyronyl, benzothiopyranyl, benzoxazinyl, benzoxazolyl, benzodioxolyl, benzodioxanyl, benzothiadiazolyl, benzotriazinyl, benzothiazolyl, benzoxazolyl, phenothioxinyl, phenothiazolyl, phenothienyl (benzothiofuranyl), phenopyronyl, phenoxazolyl, pyridinyl, dihydropyridinyl, tetrahydropyridinyl, piperidinyl, morpholinyl, thiomorpholinyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, tetrazinyl, triazolyl, benzotriazolyl, tetrazolyl, imidazolyl, pyrazolyl, thiazolyl, thiadiazolyl, isothiazolyl, oxazolyl, oxadiazolyl, pyrrolyl, furyl, dihydrofuryl, furoyl, hydantoinyl, dioxolanyl, dioxolyl, dithianyl, dithienyl, dithiinyl, thienyl, indolyl, indazolyl, benzofuryl, quinolyl, quinazolinyl, quinoxalinyl, carbazolyl, phenoxazinyl, phenothiazinyl, xanthenyl, purinyl, benzothienyl, naphtothienyl, thianthrenyl, pyranyl, pyronyl, benzopyronyl, isobenzofuranyl, chromenyl, phenoxathiinyl, indolizinyl, quinolizinyl, isoquinolyl, phthalazinyl, naphthiridinyl, cinnolinyl, pteridinyl, carbolinyl, acridinyl, perimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, imidazolinyl, imidazolidinyl, benzimidazolyl, pyrazolinyl, pyrazolidinyl, pyrrolinyl, pyrrolidinyl, piperazinyl, uridinyl, thymidinyl,cytidinyl, azirinyl, aziridinyl, diazirinyl, diaziridinyl, oxiranyl, oxaziridinyl, dioxiranyl, thiiranyl, azetyl, dihydroazetyl, azetidinyl, oxetyl, oxetanyl, thietyl, thietanyl, diazabicyclo-octyl, diazetyl, diaziridinonyl, diaziridinethionyl, chromanyl, chromanonyl, thiochromanyl, thiochromanonyl, thiochromenyl, benzofuranyl, benzisothiazolyl, benzocarbazolyl, benzochromonyl, benziso-alloxazinyl, benzocoumarinyl, thiocoumarinyl, phenometoxazinyl, phenoparoxazinyl, phentriazinyl, thiodiazinyl, thiodiazolyl, indoxyl, thio-indoxyl, benzodiazinyl (e.g. phtalazinyl), phtalidyl, phtalimidinyl, phtalazonyl, alloxazinyl, dibenzopyronyl (i.e. xanthonyl), xanthionyl, isatyl, isopyrazolyl, isopyrazolonyl, urazolyl, urazinyl, uretinyl, uretidinyl, succinyl, succinimido, benzylsultimyl, benzylsultamyl and the like, including all possible isomeric forms thereof, wherein each carbon atom of the said ring may be substituted with a substituent selected from the group consisting of halogen, nitro, C_{1-7} alkyl (optionally containing one or more functions or radicals selected from the group consisting of carbonyl (oxo), alcohol (hydroxyl), ether (alkoxy), acetal, amino, imino, oximino, alkyloximino, amino-acid, cyano, carboxylic acid ester or amide, nitro, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, C_{1-7} alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxylalkylamino, mercapto-alkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenyl-hydrazino, sulfonyl, sulfonamido and halogen), C_{3-7} alkenyl, C_{2-7} alkynyl, halo C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl, arylalkyl, alkylaryl, alkylacyl, arylacyl, hydroxyl, amino, C_{1-7} alkylamino, cycloalkylamino, alkenylamino, cyclo-alkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, hetero-cyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfhydryl, C_{1-7} alkoxy, C_{3-10} cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl,

hydroxylamino, cyano, carboxylic acid or esters or thioesters or amides thereof, thiocarboxylic acid or esters or thioesters or amides thereof; depending upon the number of unsaturations in the 3 to 10 membered ring, heterocyclic radicals may be sub-divided into heteroaromatic (or " heteroaryl") radicals and non-aromatic heterocyclic radicals; when a heteroatom of the said non-aromatic heterocyclic radical is nitrogen, the latter may be substituted with a substituent selected from the group consisting of C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl, arylalkyl and alkylaryl.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms " C_{1-7} alkoxy ", "C_{3-10} cycloalkoxy ", " aryloxy", "arylalkyloxy ", " oxyheterocyclic ", "thio C_{1-7} alkyl", " thio C_{3-10} cycloalkyl ", " arylthio ", " arylalkylthio " and " thioheterocyclic" refer to substituents wherein a C_{1-7} alkyl radical, respectively a C_{3-10} cycloalkyl, aryl, arylalkyl or heterocyclic radical (each of them such as defined herein), are attached to an oxygen atom or a sulfur atom through a single bond, such as but not limited to methoxy, ethoxy, propoxy, butoxy, thioethyl, thiomethyl, phenyloxy, benzyloxy, mercaptobenzyl, cresoxy and the like.

As used herein with respect to a substituting atom, and unless otherwise stated, the term halogen means any atom selected from the group consisting of fluorine, chlorine, bromine and iodine.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " halo C_{1-7} alkyl " means a C_{1-7} alkyl radical (such as above defined) in which one or more hydrogen atoms are independently replaced by one or more halogens (preferably fluorine, chlorine or bromine), such as but not limited to difluoromethyl, trifluoromethyl, trifluoroethyl, octafluoropentyl, dodecafluoroheptyl, dichloromethyl and the like; the term "halo C_{1-4} alkyl " designate the corresponding radical with only 1 to 4 carbon atoms, and so on.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms " C_{2-7} alkenyl " and " aliphatic unsaturated hydrocarbon radical with 2 to 7 carbon atoms " are interchangeable and designate a straight and branched acyclic hydrocarbon monovalent radical having one or more ethylenical unsaturations and having from 2 to 7 carbon atoms such as, for example, vinyl, 2-propenyl, 3-butenyl, 2-butenyl, 2-pentenyl, 3-pentenyl, 3-methyl-2-butenyl, 3-hexenyl, 2-hexenyl, 2-heptenyl, butadienyl, pentadienyl, hexadienyl, heptadienyl, heptatrienyl and the like, including all possible isomers thereof; the term " C_{3-7} alkenyl " designate the corresponding radical with only 3 to 7 carbon atoms, and so on.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms " C_{3-10} cycloalkenyl" and " cycloaliphatic unsaturated hydrocarbon radical with 3 to 10 carbon atoms " are interchangeable and mean a monocyclic mono- or polyunsaturated hydrocarbon monovalent radical having from 3 to 8 carbon atoms, such as for instance cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, cyclohexadienyl, cycloheptenyl, cycloheptadienyl, cyclohepta-trienyl, cyclooctenyl, cyclooctadienyl and the like, or a C_{7-10} polycyclic mono- or polyunsaturated hydrocarbon mono-valent radical having from 7 to 10 carbon atoms such as dicyclopentadienyl, fenchenyl (including all isomers thereof, such

as α-pinolenyl), bicyclo[2.2.1]hept-2-enyl, bicyclo[2.2.1]hepta-2,5-dienyl, cyclo-fenchenyl and the like.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " C_{2-7} alkynyl " defines straight and branched chain hydrocarbon radicals containing one or more triple bonds and having from 2 to 20 carbon atoms such as, for example, acetylenyl, 2-propynyl, 3-butynyl, 2-butynyl, 2-pentynyl, 3-pentynyl, 3-methyl-2-butynyl, 3-hexynyl, 2-hexynyl and the like and all possible isomers thereof.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms " arylalkyl " and "heterocyclic-substituted alkyl" refer to an aliphatic saturated hydrocarbon monovalent radical, preferably a C_{1-7} alkyl or a C_{3-10} cycloalkyl such as defined above, onto which an aryl radical or respectively a heterocyclic radical (such as defined above) is already bonded, such as but not limited to benzyl, pyridylmethyl, pyridylethyl, 2-(2-pyridyl)isopropyl, oxazolylbutyl, 2-thienylmethyl and 2-furylmethyl.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " alkylaryl " and "alkyl-substituted heterocyclic" refer to an aryl radical or respectively a heterocyclic radical (such as defined above) onto which is (are) already bonded one or more aliphatic saturated hydrocarbon monovalent radicals, preferably C_{1-7} alkyl radicals or C_{3-10} cycloalkyl radicals as defined above such as, but not limited to, o-toluyl, m-toluyl, p-toluyl, mesityl and 2,4,6-trimethylphenyl.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms " alkylamino ", "cycloalkylamino ", "alkenyl-amino", " cycloalkenylamino " , " arylamino ", "arylalkylamino", "heterocyclic amino " , " hydroxyalkylamino ", "mercaptoalkylamino " and " alkynylamino" mean that respectively one (thus monosubstituted amino) or even two (thus disubstituted amino) C_{1-7} alkyl, C_{3-10} cycloalkyl, C_{2-7} alkenyl, C_{3-10} cycloalkenyl, aryl, arylalkyl, heterocyclic, mono- or polyhydroxy C_{1-7} alkyl, mono- or polymercapto C_{1-7} alkyl or C_{2-7} alkynyl radical(s) (each of them as defined herein, respectively) is/are attached to a nitrogen atom through a single bond or, in the case of heterocyclic, include a nitrogen atom, such as but not limited to, anilino, benzylamino, methylamino, dimethylamino, ethylamino, diethylamino, isopropylamino, propenylamino, n-butylamino, ter-butylamino, dibutylamino, morpholino-alkylamino, morpholinyl, piperidinyl, piperazinyl, hydroxymethylamino, β-hydroxyethylamino and ethynylamino; this definition also includes mixed disubstituted amino radicals wherein the nitrogen atom is attached to two such radicals belonging to two different sub-set of radicals, e.g. an alkyl radical and an alkenyl radical, or to two different radicals within the same sub-set of radicals, e.g. methylethylamino; the term " C_{3-7} alkyl-amino" designates the corresponding radical with only 3 to 7 carbon atoms in the alkyl group(s) attached to nitrogen, for instance di-isopropylamino, and so on; among disubstituted amino radicals, symetrically substituted are usually preferred and more easily accessible.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms "(thio)carboxylic acid ester " , " (thio)carboxylic acid thioester " and " (thio)carboxylic acid amide" refer to radicals wherein the carboxyl or thiocarboxyl group is directly attached to

the pteridine ring (e.g. in the 6- and/or 7-position) and wherein said carboxyl or thiocarboxyl group is bonded to the hydrocarbonyl residue of an alcohol, a thiol, a polyol, a phenol, a thiophenol, a primary or secondary amine, a polyamine, an amino-alcohol or ammonia, the said hydrocarbonyl residue being selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, arylalkyl, alkylaryl, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, arylamino, arylalkylamino, heterocyclic amino, hydroxyalkylamino, mercapto-alkylamino or alkynylamino (such as above defined, respectively).

As used herein with respect to a substituting radical, and unless otherwise stated, the term " amino-acid " refers to a radical derived from a molecule having the chemical formula H_2N–CHR–COOH, wherein R is the side group of atoms characterizing the amino-acid type; said molecule may be one of the 20 naturally-occurring amino-acids or any similar non naturally-occurring amino-acid.

As used herein and unless otherwise stated, the term " stereoisomer " refers to all possible different isomeric as well as conformational forms which the compounds of formula (I) or (II) may possess, in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers and/or conformers of the basic molecular structure. Some compounds of the present invention may exist in different tautomeric forms, all of the latter being included within the scope of the present invention.

As used herein and unless otherwise stated, the term " enantiomer " means each individual optically active form of a compound of the invention, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (i.e. at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

As used herein and unless otherwise stated, the term " solvate " includes any combination which may be formed by a pteridine derivative of this invention with a suitable inorganic solvent (e.g. hydrates) or organic solvent, such as but not limited to alcohols, ketones, esters and the like.

As used herein and unless otherwise stated, the terms " dihydro-pteridine derivative " and " tetrahydropteridine derivative " refer to the hydrogenation products of the pteridine derivatives having formula (I) or (II), i.e. derivatives wherein two hydrogen atoms are present in positions 5 and 6, or 7 and 8, of the pteridine ring, or wherein four hydrogen atoms are present in positions 5, 6, 7 and 8 of the said ring; such hydrogenated derivatives are easily accessible from the pteridine derivatives using hydrogenation methods well known in the art.

DETAILED DESCRIPTION OF THE INVENTION

A main object of the invention is to provide a pharmaceutical composition having high immunosuppressive activity. Thus, the present invention relates in particular to the medical applications of a group of pteridine derivatives, their pharmaceutically acceptable salts, N-oxides, solvates, dihydro- and tetrahydroderivatives and enantiomers, possessing unexpectedly desirable pharmaceutical properties, in particular which are highly active

immunosuppressive agents, and as such are useful in the treatment in transplant rejection and/or in the treatment of certain inflammatory diseases.

Surprisingly, the compounds of the present invention show a broader therapeutic spectrum profile than merely immunosuppressive activity, as is evidenced by the results obtained in the diversity of test procedures disclosed hereinbelow. A further advantageous feature of the compounds of the present invention resides in their excellent oral activity.

In the first embodiment of the invention, the novel pteridine derivatives are as defined in the general formula (I), wherein each of the substituents X, Z, R_1, R_2, R_3 and R_4 may correspond to any of the definitions given above (and, when X includes sulfur, wherein m may be 0, 1 or 2), in particular with any of the individual meanings (such as illustrated above) of generic terms such as, but not limited to, "C_{1-7} alkyl ", " C_{2-7} alkenyl ", " C_{2-7} alkynyl ", " aryl ", " alkylaryl ", "arylalkyl ", "alkylamino", " cycloalkylamino ", "alkenylamino ", "alkynylamino", "arylamino", " arylalkylamino ", " C_{1-7} alkoxy", " C_{3-10} cycloalkoxy ", "thio C_{1-7} alkyl ", " thio C_{3-10} cycloalkyl ", " halo C_{1-7} alkyl ", "amino-acid " and the like.

When a mixture of enantiomers of a pteridine derivative having the general formula (I) according to the invention is obtained during synthesis, the said mixture may be separated by means and methods standard in the art, e.g. liquid chromatography using one or more suitable chiral stationary phases. The latter include, for example, polysaccharides, in particular cellulose or amylose derivatives. Commercially available polysaccharide-based chiral stationary phases suitable for this purpose are ChiralCel™ CA, OA, OB, OC, OD, OF, OG, OJ and OK, and Chiralpak™ AD, AS, OP(+) and OT(+). Appropriate eluents or mobile phases for use in combination with said polysaccharide-based chiral stationary phases are hydrocarbons such as hexane and the like, optionally admixed with an alcohol such as ethanol, isopropanol and the like. The above mixture of enantiomers may alternatively be separated by forming diastereoisomers, followed by separation of the diastereoisomers, e.g. by differential crystallization or chromatography. The resolving agent may be cleaved from the separated diastereoisomers, e.g. by treatment with acids or bases, in order to generate the pure enantiomers of the compounds of the invention.

Some preferred pteridine derivatives having the general formula (I) or (II) according to the invention are more specifically illustrated in the following examples and defined in the following claims. For instance, useful pteridine species disclosed below include those wherein:

- R_1 is selected from the group consisting of methyl, ethyl, isopropyl, pentyl and benzyl, and/or
- R_2 is amino, and/or
- R_4 is hydrogen or methoxy, and/or
- R_3 is 3-thienyl or a phenyl group with one or more substituents (in the latter case, such substituents are preferably each independently selected from the group consisting of fluoro, methoxy, ethoxy, trifluoromethyl, dimethylamino, chloro, cyano, methyl, ethyl,

carboxymethyl, methylthio, dimethylcarboxamido, diethylcarboxamido and methylcarboxylate, and/or

- X is a sulfur atom (i.e. m is 0) or an oxygen atom, or
- X is NZ, wherein Z is selected from the group consisting of hydrogen, methyl, ethyl, isopropyl and benzyl, or NZ together with R_1 forms a radical selected from the group consisting of hydroxylamino, morpholinyl, piperidinyl, piperazinyl, N-methylpiperazinyl, 1,2,4-triazolyl and pyrrolidinyl.

The present invention further provides processes and methods for making the novel pteridine derivatives having the general formula (I). As a general rule, the preparation of these compounds is based on the principle that, starting from a suitable pteridine precursor, each of the substituents XR_1, R_2, R_3 and R_4 may be introduced separately (except, of course, when R_3 together with R_4 forms a homocyclic or heterocyclic radical) without adversely influencing the presence of one or more substituents already introduced at other positions on the pteridine ring or the capacity to introduce further substituents later on.

Methods of manufacture have been developed by the present inventors which may be used alternatively to, or may be combined with, the methods of synthesis already known in the art of pteridine derivatives (depending upon the targeted final compound). For instance, methods for simultaneously introducing R_3 and R_4 in the form of a homocyclic or heterocyclic radical at positions 6 and 7 of the pteridine ring are already known from U.S. Patent No. 2,581,889. The synthesis of mono- and di-*N*-oxides of the pteridine derivatives of this invention can easily be achieved by treating the said derivatives with an oxidizing agent such as, but not limited to, hydrogen peroxide (e.g. in the presence of acetic acid) or a peracid such as chloroperbenzoic acid. Dihydro- and tetrahydropteridine derivatives of this invention can easily be obtained by catalytic hydrogenation of the corresponding pteridine derivatives, e.g. by placing the latter in a hydrogen atmosphere in the presence of platinum oxide or platinum. The methods for making the pteridine derivatives of the present invention will now be explained in more details by reference to the appended figures 1 to 8 wherein, unless otherwise stated hereinafter, each of the substituting groups or atoms X, Z, R_1, R_2, R_3 and R_4 is as defined in formula (I) of the summary of the invention and, more specifically, may correspond to any of the individual meanings disclosed above. The same manufacturing methods may also be applied, if need be, while starting from pteridine derivatives which are already known in the art. In the description of the reaction steps involved in each figure, reference is made to the use of certain catalysts and/or certain types of solvents. It should be understood that each catalyst mentioned should be used in a catalytic amount well known to the skilled person with respect to the type of reaction involved. Solvents that may be used in the following reaction steps include various kinds of organic solvents such as protic solvents, polar aprotic solvents and non-polar solvents as well as aqueous solvents which are inert under the relevant reaction conditions. More specific examples include aromatic hydrocarbons, chlorinated hydrocarbons, ethers, aliphatic hydrocarbons, alcohols, esters, ketones, amides, water or mixtures thereof, as well as supercritical solvents such as carbon

dioxide (while performing the reaction under supercritical conditions). The suitable reaction temperature and pressure conditions applicable to each kind of reaction step will not be detailed herein but do not depart from the relevant conditions already known to the skilled person with respect to the type of reaction involved and the type of solvent used (in particular its boiling point).

Figure 1 represents a scheme for the preparation of 2,4,6-trisubstituted pteridines with various R_2 and R_3 substituents in the 2- and 6- positions of the pteridine ring, respectively. In the first step (a), a chloropyrimidine **1**, wherein R_2 may be *inter alia* amino, alkylamino, arylamino, alkoxy, aryloxy, mercaptoalkyl, or mercaptoaryl, is reacted with an appropriate nucleophile R_1XH, the said nucleophile being selected from the group consisting of alcohols (e.g. methanol, ethanol, isopropanol or benzylalcohol), thiols, primary amines and secondary amines wherein R_1 may be *inter alia* alkyl, cycloalkyl, aryl, alkylaryl, heteroaryl or alkylheteroaryl. Introduction of a nitroso group into the pyrimidine intermediate **2** occurs in step (b) under acidic aqueous conditions in the presence of sodium nitrite $NaNO_2$. Reduction of the nitroso functionality of the pyrimidine intermediate **3** into a free amino group in intermediate **4** is then effected in step (c) by means of reducing agents (such as $Na_2S_2O_4$ or $(NH_4)_2S$) in water, or catalytically (Pt/H_2) in the presence of a protic solvent. In step (d), ring closure is performed by treating the diaminopyrimidine **4** with glyoxal in order to form a pteridine ring. In step (e), the nitrogen atom at position 8 of the pteridine ring of compound **5** is oxidized, e.g. using H_2O_2 under acidic conditions. In step (f), a chlorine atom is regioselectively introduced on the 6 position of the pteridine ring of compound **6** by treatment with a carboxylic acid choride such as acetyl chloride under acidic conditions. Then in step (g) the 6-chlorosubstituted pteridine **7** is reacted with a boronic acid having the general formula $R_3B(OH)_2$, wherein R_3 may be alkyl, cycloalkyl, aryl or heteroaryl, under basic conditions (such as in the presence of an aqueous alcaline solution) and a palladium based catalyst, thus yielding the desired derivative **8** of the present invention.

Figure 2 represents a scheme for the preparation of 2,4,6- or 2,4,7-trisubstituted or 2,4,6,7-tetrasubstituted pteridine derivatives with various R_1, R_2, R_3 and/or R_4 substituents. In step (a), the thiol function of 2-mercapto-4,6-diaminopyrimidine is alkylated, preferably methylated by reaction with methyl iodide in the presence of a solvent such as ethanol, in order to yield 2-thiomethyl-4,6-diaminopyrimidine. Introduction of a nitroso group in the 5-position of the pyrimidine ring is then achieved in step (b) by using sodium nitrite under aqueous acidic conditions. In step (c), the methylthio group in the 2-position is exchanged for a group R_2 by reaction with an appropriate nucleophile, wherein R_2 is as defined above and preferably is primary or secondary amino, C_{1-7} alkoxy, aryloxy, C_{3-10} cycloalkoxy, heteroaryloxy, mercapto C_{1-7}alkyl, mercaptoaryl, mercapto C_{3-10}cycloalkyl or mercapto-heteroaryl. Reduction of the nitroso group is then achieved in step (d) either catalytically (Pt/H_2) in the presence of a protic solvent or chemically using sodium dithionite or ammonium sulfide in the presence of water. Then in step (e), the resulting 2-R_2-substituted-4,5,6-triaminopyrimidine is condensed, under acidic condi-tions in the presence of a solvent such as

methanol, with an α-ketoaldoxime bearing the group R_3, wherein R_3 may be C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl or heteroaryl, into a 2,6-substituted-4-aminopteridine derivative. Alternatively, the corresponding 2,7-substituted-4-aminopteridine derivative can be obtained in step (f) by reacting the 2-R_2-substituted-4,5,6-triaminopyrimidine with a monosubstituted glyoxal bearing a group R_4, wherein R_4 may be inter alia C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl or heteroaryl. Alternatively, a 2-R_2-substituted 4-amino-6,7-disubstituted pteridine derivative can be obtained in step (g) by reacting the 2-R_2-substituted 4,5,6-triaminopyrimidine with a disubstituted glyoxal bearing groups R_3 and R_4, wherein each of R_3 and R_4 is independently selected (i.e. R_3 and R_4 may be identical or different) from the group consisting of C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl and heteroaryl, under neutral or basic conditions. In step (h), acidic or basic hydrolysis of the amino group at position 4 of the pteridine ring is performed and results in the corresponding 4-oxopteridine derivative. In step (i), the hydroxyl group of the tautomeric form of the latter is activated by nucleophilic displacement, e.g. by preparing the 4-[(1,2,4)-triazolyl] pteridine derivative. Finally in a first part of step (j), a nucleophilic displacement is performed by mixing the said 4-triazolylpteridine derivative with a nucleophile having the general formula R_1XH, such as for example a primary or secondary amine, C_{1-7} alkoxy, aryloxy, C_{3-10} cycloalkoxy, heteroaryloxy, mercapto C_{1-7} alkyl, mercaptoaryl, mercapto C_{3-10} cycloalkyl, or mercapto-heteroaryl yielding the desired final pteridine derivatives.

Figure 3 represents a scheme for the preparation of 2,4,6- or 2,4,7-trisubstituted or 2,4,6,7-tetrasubstituted pteridine derivatives with various substituents on the pteridine ring, starting from the 2-thiomethyl-5-nitroso-4,6-diaminopyrimidine obtained after step (b) of the scheme shown in figure 2. Reduction of the nitroso group is achieved in step (a) either catalytically (Pt/H_2) in the presence of a protic solvent or chemically using sodium dithionite or ammonium sulfide in the presence of water. Then in step (b), 2-thiomethyl-4,5,6-triamino-pyrimidine is condensed, under acidic conditions in the presence of a solvent such as methanol, with an α-ketoaldoxime bearing the group R_3, wherein R_3 may be *inter alia* C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl or heteroaryl, thus regioselectively yielding a 2-thiomethyl-4-amino-6-R_3-substituted-pteridine derivative. Alternatively, the corresponding 2-thiomethyl-4-amino-7-R_4-substituted pteridine is obtained in step (c) by reacting 2-thiomethyl-4,5,6-triamino-pyrimidine with a monosubstituted glyoxal bearing a group R_4, wherein R_4 may be *inter alia* C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl or heteroaryl. Alternatively, the corresponding 2-thiomethyl-4-amino-6-R_3-7-R_4-substituted pteridine is obtained in step (d) by reacting 2-thiomethyl-4,5,6-triamino-pyrimidine with a disubstituted glyoxal bearing groups R_3 and R_4, wherein each of R_3 and R_4 is independently selected (i.e. R_3 and R_4 may be identical or different) from the group consisting of C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl and heteroaryl, under neutral or basic conditions. In step (e), the methylthio group in the 2-position is oxidized to the corresponding sulfone by using oxidizing agents such as chloroperoxybenzoic acid in chloroform or hydrogen peroxide in acetic acid. The methylsulfonyl group is easily exchanged in step (f) by reaction with a nucleophile, such as for example a primary or secondary amine, C_{1-7} alkoxy, aryloxy, C_{3-10} cycloalkoxy, heteroaryloxy, mercapto C_{1-7} alkyl, mercaptoaryl, mercapto C_{3-10} cycloalkyl, or

mercapto-heteroaryl. In step (g), acidic or basic hydrolysis of the amino group at position 4 of the pteridine ring is performed and results in the corresponding 4-oxopteridine derivative. In step (h), the hydroxyl group of the tautomeric form of the latter is activated by nucleophilic displacement, e.g. by preparing the 4-[(1,2,4)-triazolyl] pteridine derivative. In the last step (i), a nucleophilic displacement is performed by mixing the said 4-triazolylpteridine derivative with a nucleophile having the general formula R_1XH, such as for example a primary or secondary amine, C_{1-7} alkoxy, aryloxy, C_{3-10} cycloalkoxy, heteroaryloxy, mercapto C_{1-7} alkyl, mercaptoaryl, mercapto C_{3-10} cycloalkyl, or mercapto-heteroaryl.

Figure 4 represents a scheme for the synthesis of unsymmetrical 2,4,6-trisubstituted and 2,4,7-trisubstituted, as well as 2,4,6,7-tetrasubstituted, pteridine derivatives with various R_1, R_2, R_3 and/or R_4 substituents in the 2-, 4-, 6- and/or 7-positions of the pteridine ring, respectively. In step (a), a 2-R_2-substituted-4,5,6-triamino-pyrimidine is condensed with an α-keto-aldoxime bearing a radical R_3, wherein R_3 may be *inter alia* selected from the group consisting of C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl and heteroaryl, in a protic solvent such as methanol under acidic conditions yielding regioselectively a 4-amino-pteridine bearing a R_2-substituent in position 2 and a R_3 substituent in position 6 of the pteridine ring. Alternatively, a 2-R_2-substituted-4-amino-7-R_4-substituted pteridine derivative can be obtained in step (b) by reacting a 2-R_2-substituted-4,5,6-triamino-pyrimidine with a monosubstituted glyoxal bearing the group R_4, wherein R_4 may be *inter alia* selected from the group consisting of C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl and heteroaryl, under neutral or basic conditions. Alternatively, a 2-R_2-substituted-4-amino-6,7-di-substituted pteridine derivative can be obtained in step (c) by reacting a 2-R_2-substituted-4,5,6-triamino-pyrimidine with a disubstituted glyoxal bearing the groups R_3 and R_4, wherein each of R_3 and R_4 is independently selected (i.e. R_3 and R_4 may be identical or different) from the group consisting of C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl or heteroaryl, under neutral or basic conditions. In step (d), acidic or basic hydrolysis of the amino group at position 4 of the pteridine ring is performed and results in the corresponding 4-oxo-pteridine derivative. In step (e), the hydroxyl group of the tautomeric form of the latter is activated for a nucleophilic displacement reaction, e.g. by preparing the 4-[(1,2,4)-triazolyl]pteridine derivative. In the last step (f), the 4-triazolylpteridine derivative is reacted with a nucleophile having the general formula R_1XH, such as for example a primary or secondary amine, C_{1-7} alkoxy, aryloxy, C_{3-10} cycloalkoxy, heteroaryloxy, mercapto C_{1-7} alkyl, mercaptoaryl, mercapto C_{3-10} cycloalkyl, or mercapto-heteroaryl.

Figure 5 represents a scheme for the synthesis of symmetrical 2,4,6-trisubstituted and 2,4,7-trisubstituted, as well as 2,4,6,7-tetrasubstituted pteridine derivatives with various R_1, R_2, R_3 and/or R_4 substituents in the 2-, 4-, 6- and/or 7-positions of the pteridine ring. In step (a), a nitroso group is introduced on position 5 of the pyrimidine ring of a 2-R_2-substituted 4-oxo-6-aminopyrimidine by using sodium nitrite under aqueous acidic conditions. Reduction of the nitroso group in step (b) is achieved either catalytically (Pt/H_2) in the presence of a protic solvent, or chemically using sodium dithionite or ammonium sulfide in water. Then in a next step (c), condensing the resulting 2-R_2-substituted 4-oxo-5,6-diamino-pyrimidine with an α-

ketoaldoxime bearing a radical R_3, wherein R_3 may be *inter alia* C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl or heteroaryl, in a protic solvent such as methanol under acidic conditions regioselectively yields a 4-oxopteridine bearing a R_2 substituent in position 2 and a R_3 substituent in position 6 of the pteridine ring. Alternatively, a 2-R_2-substituted 4-oxo-7-R_4-substituted pteridine derivative can be obtained in step (d) by reacting the 2-R_2-substituted 4-oxo-5,6-diamino-pyrimidine with a monosubstituted glyoxal bearing the group R_4, wherein R_4 may be *inter alia* C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl or heteroaryl, under neutral or basic conditions. Alternatively, a 2-R_2-substituted-4-oxo-6,7-disubstituted pteridine derivative can be obtained in step (e) by reacting the 2-R_2-substituted 4-oxo-5,6-diamino-pyrimidine with a disubstituted glyoxal bearing groups R_3 and R_4, wherein each of R_3 and R_4 is independently selected (i.e. R_3 and R_4 may be identical or different) from the group consisting of C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl and heteroaryl, under neutral or basic conditions. Activation of the (tautomeric) hydroxyl substituent in position 4 of the pteridine ring for a nucleophilic displacement reaction occurs in step (f) by preparing the corresponding 4-[(1,2,4)-triazolyl] pteridine derivative, e.g. using $POCl_3$ or 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine as solvent. When R_2 is an amino group, protection of R_2 may further be necessary before carrying out this reaction. The amino group can be protected for instance by an acetyl group, which can be hydrolysed back to the amino group in a next step. Nucleophilic substitution is performed in step (g) by mixing the triazolyl pteridine derivative with a nucleophile R_1XH, (such as for example such as for example a primary or secondary amine, C_{1-7} alkoxy, aryloxy, C_{3-10} cycloalkoxy, heteroaryloxy, mercapto C_{1-7} alkyl, mercaptoaryl, mercapto C_{3-10} cycloalkyl, or mercapto-heteroaryl) at room temperature in a polar aprotic solvent such as 1,4-dioxane.

Figure 6 represents a scheme for the preparation of symmetrical 2,4,6- trisubstituted pteridines and 2,4,7-trisubstituted as well as 2,4,6,7-tetrasubstituted pteridine derivatives, i.e. wherein substituents in the 2- and 4- positions of the pteridine ring are identical. In step (a), a nitro group is introduced in position 5 of a 6-amino-2,4-dioxopyrimidine under strongly acidic conditions (e.g. HNO_3, H_2SO_4). Then, in step (b), both hydroxyl groups from the tautomeric form are converted to chloro groups by treatment with a chlorinating agent such as $POCl_3$ or $SOCl_2$. Both chloro groups are then displaced in step (c) with a nucleophile having the general formula R_1XH. The nitro group of the latter is then reduced in step (d) to an amino group by treatment with a reducing agent (e.g. Pt/H_2). Finally, reaction of the latter with an α-ketoaldoxime bearing the group R_3, wherein R_3 may be *inter alia* aryl, C_{1-7} alkyl, C_{1-10} cycloalkyl or heteroaryl, regioselectively yields the desired 2,4,6-trisubstituted pteridine derivative in step (e). Alternatively, reaction of the 2,4-substituted-5,6-diaminopyrimidine from step (d) with a monosubstituted glyoxal bearing a group R_4 wherein R_4 may be *inter alia* C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl or heteroaryl yields the desired 2,4,7-trisubstituted pteridine derivative in step (f). Alternatively, reaction of the 2,4-substituted-5,6-diaminopyrimidine from step (d) with a disubstituted glyoxal bearing groups R_3 and R_4, wherein each of R_3 and R_4 is independently selected (i.e. R_3 and R_4 may be identical or different) from the group consisting

of C_{1-7} alkyl, C_{3-10} cycloalkyl, aryl and heteroaryl, under neutral or basic conditions, yields the desired 2,4,6,7-tetrasubstituted pteridine derivative in step (g).

Figure 7 represents a scheme for the preparation of 2,4,6-trisubstituted pteridine derivatives, wherein the substituent in the 6- position of the pteridine ring is a phenyl group substituted by a nitrogen-containing function. In step (a), the chlorine at position 4 of the pyrimidine ring is displaced by an appropriate nucleophile, having the general formula R_1XH (such as, but not limited to, a primary or secondary amine, C_{1-7} alkoxy, aryloxy, C_{3-10} cycloalkoxy, heteroaryloxy, mercapto C_{1-7} alkyl, mercaptoaryl, mercapto C_{3-10} cycloalkyl, or mercapto-heteroaryl). Introduction of the nitroso group at position 5 of the pyrimidine ring is achieved by treatment with sodium nitrite under aqueous acidic conditions. Reduction of the nitroso group in step (c) is achieved either catalytically (Pt/H_2) in the presence of a protic solvent, or chemically using sodium dithionite or ammonium sulfide in water. In step (d), the 2-R_2-4-XR_1-substituted-5,6-triamino-pyrimidine analogue is condensed with an acetamidophenylglyoxalmonoxime in a protic solvent such as methanol under acidic conditions to yield regioselectively a pteridine analogue bearing an acetamidophenyl group at position 6 of the pteridine ring. Acidic deprotection of the acetyl group leads to the formation of the free amino group. This amino group can be transformed into amides (by reaction with carboxylic acids or acid chlorides) or into sulfonamides (by reaction with sulfonyl chlorides) by reaction in a polar aprotic solvent (e.g. pyridine, dimethylformamide or dichloromethane) and optionally further in the presence of a base (e.g. triethylamine).

Figure 8 represents a scheme for the preparation of 2,4,6-trisubstituted pteridine derivatives, wherein the substituent in the 6- position of the pteridine ring is a phenyl group substituted by an oxygen-containing function. In step (a) a 2-R_2-4-XR_1-substituted-5,6-triaminopyrimidine, which may be obtained for instance after step (c) described in figure 7, is condensed with an hydroxyphenyl-glyoxalmonoxime in a protic solvent such as methanol under acidic conditions to yield regioselectively a pteridine analogue bearing an hydroxyphenyl group at position 6 of the pteridine ring. The free hydroxyl group can be alkylated in a polar protic solvent (e.g. dimethylformamide) using a base (such as, but not limited to, potassium carbonate, cesium carbonate or sodium hydride) and an appropriate alkylhalide or arylalkylhalide.

Some sub-sets of pteridine derivatives according to the invention deserve specific interest. Thus in a particular embodiment the invention relates to a group of pteridine derivatives, as well as pharmaceutical compositions comprising such pteridine derivatives as active principle, having the above general formula (I) wherein:

- X is NZ,
- XR_1 is selected from the group consisting of hydroxylamino; hydrazino (i.e. Z is hydrogen and R_1 is amino); (mono- or di-) C_{1-7} alkylamino (i.e. R_1 is C_{1-7} alkyl); (mono- or di-) arylamino (i.e. R_1 is aryl, e.g. adamantyl); (mono- or di-) C_{3-10} cycloalkylamino (i.e. R_1 is C_{3-10} cycloalkyl); (mono- or di-) hydroxyC_{1-7} alkylamino (i.e. R_1 is C_{1-7} alkyl substituted with hydroxyl); (mono- or di-) C_{1-4} alkylarylamino; and saturated or unsaturated

heterocyclic groups containing at least one nitrogen atom and optionally substituted by one or more substituents independently selected from the group consisting of C_{1-4} alkyl, hydroxy C_{1-4} alkyl, C_{1-4} alkyloxy, halogen, hydroxy, hydroxycarbonyl and C_{1-4} alkyloxycarbonyl, such as but not limited to piperazinyl, N-alkylpiperazinyl and morpholinyl;

- R_2 is amino or is selected from the group consisting of hydroxylamino; hydrazino (i.e. Z is hydrogen and R_1 is amino); (mono- or di-) arylamino (i.e. R_1 is aryl); (mono- or di-) C_{3-10} cycloalkylamino (i.e. R_1 is C_{3-10} cycloalkyl); (mono- or di-) hydroxyC_{1-7} alkylamino (i.e. R_1 is C_{1-7} alkyl substituted with hydroxyl); (mono- or di-) C_{1-4} alkylarylamino; and saturated or unsaturated heterocyclic groups containing at least one nitrogen atom and optionally substituted by one or more substituents independently selected from the group consisting of C_{1-4} alkyl, hydroxy C_{1-4} alkyl, C_{1-4} alkyloxy, halogen, hydroxy, hydroxycarbonyl and C_{1-4} alkyloxycarbonyl, such as but not limited to piperazinyl, N-alkylpiperazinyl and morpholinyl;
- R_3 is selected from the group consisting of unsubstituted, monosubstituted and disubstituted aryl groups (wherein the substituent(s) may indepen-dently be halogen, C_{1-4} alkoxy or C_{1-4} alkyl); 3,4,5-trimethoxyphenyl; 3,4-formylidene-3,4-dihydroxyphenyl; aryl groups bonded to the pteridine ring via a saturated or unsaturated aliphatic spacer which may be halogenated or hydroxylated; and aliphatic substituents which may contain ether function, alcohol function, or substituted or unsubstituted amino functions or C_{1-4} alkyloxy; and
- R_4 is selected from the group consisting of hydrogen, alkyl and alkoxy;

or a pharmaceutically acceptable addition salt or a stereochemical isomeric form thereof.

In another particular embodiment, the invention relates to a group of pteridine derivatives, as well as pharmaceutical compositions comprising such pteridine derivatives as active principle, having the above general formula (I) wherein:

- X is oxygen or sulfur;
- R_1 is C_{1-7} alkyl;
- R_2 is amino or is selected from the group consisting of hydroxylamino; hydrazino (i.e. Z is hydrogen and R_1 is amino); (mono- or di-) arylamino (i.e. R_1 is aryl); (mono- or di-) C_{3-10} cycloalkylamino (i.e. R_1 is C_{3-10} cycloalkyl); (mono- or di-) hydroxyC_{1-7} alkylamino (i.e. R_1 is C_{1-7} alkyl substituted with hydroxyl); (mono- or di-) C_{1-4} alkylarylamino; and saturated or unsaturated heterocyclic groups containing at least one nitrogen atom and optionally substituted by one or more substituents independently selected from the group consisting of C_{1-4} alkyl, hydroxy C_{1-4} alkyl, C_{1-4} alkyloxy, halogen, hydroxy, hydroxycarbonyl and C_{1-4} alkyloxycarbonyl, such as but not limited to piperazinyl, N-alkylpiperazinyl and morpholinyl;
- R_3 is selected from the group consisting of unsubstituted, monosubstituted and disubstituted aryl groups (wherein the substituent(s) may indepen-dently be halogen, C_{1-4} alkoxy or C_{1-4} alkyl); aryl groups bonded to the pteridine ring via a saturated or

unsaturated aliphatic spacer which may be halogenated or hydroxylated; and aliphatic substituents which may contain ether function, alcohol function, or substituted or unsubstituted amino functions or C_{1-4} alkyloxy; and

- R_4 is selected from the group consisting of hydrogen, alkyl and alkoxy;

or a pharmaceutically acceptable addition salt or a stereochemical isomeric form thereof.

In another particular embodiment, the invention relates to a group of pteridine derivatives having the above general formula (I) wherein X represents an oxygen atom or a group with the formula $S(O)_m$, wherein m is an integer from 0 to 2, or a group with the formula NZ and wherein:

- R_1 is a group selected from the group consisting of C_{1-4} alkyl, C_{2-7} alkenyl, C_{2-7} alkynyl, C_{3-10} cycloalkyl, C_{3-10} cycloalkenyl, aryl, alkylaryl, arylalkyl, heterocyclic, heterocyclic-substituted alkyl and alkyl-substituted heterocyclic, each of said groups being optionally substituted with one or more substituents selected from the group consisting of halogen, C_{1-4} alkyl, C_{1-4} alkoxy, C_{2-7} alkenyl, C_{2-7} alkynyl, halo C_{1-4} alkyl, C_{3-10} cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, hydroxyl, sulfhydryl, nitro, hydroxylamino, mercaptoamino, cyano, carboxylic acid or esters or thioesters or amides or thioamides or halides or anhydrides thereof, thiocarboxylic acid or esters or thioesters or amides or thioamides or halides or anhydrides thereof, carbamoyl, thiocarbamoyl, ureido, thio-ureido, amino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkyl-amino, hydroxylalkylamino, mercaptoalkyl-amino, heterocyclic amino, hydrazino, alkylhydrazino and phenyl-hydrazino; or R_1 is a carboxyalkyl, carboxyaryl, thiocarboxyaryl or thiocarboxyalkyl group;
- Z is a group independently defined as R_1 or Z is hydrogen or the group NZ together with R_1 is either hydroxylamino or an optionally substituted heterocyclic group containing at least one nitrogen atom;
- R_2 is selected from the group consisting of amino; acylamino; thioacylamino; carbamoyl; thiocarbamoyl, ureido; thio-ureido, sulfonamido; hydroxylamino; alkoxyamino; thioalkylamino; mercaptoamino, hydrazino; alkylhydrazino; phenylhydrazino; optionally substituted heterocyclic radicals; C_{3-7} alkylamino; arylamino; arylalkyl-amino; cycloalkylamino; alkenylamino; cycloalkenylamino; heterocyclic amino; hydroxyalkylamino; mercaptoalkylamino; C_{1-7} alkoxy; C_{3-10} cycloalkoxy; thio C_{1-7} alkyl; arylsulfoxide; arylsulfone; heterocyclic sulfoxide; heterocyclic sulfone; thio C_{3-10} cycloalkyl; aryloxy; arylthio; arylalkyloxy; arylalkylthio; oxyheterocyclic and thioheterocyclic radicals;
- R_4 is an atom or a group selected from the group consisting of hydrogen; halogen; C_{1-7} alkyl; C_{2-7} alkenyl; C_{2-7} alkynyl; halo C_{1-7} alkyl; carboxy C_{1-7} alkyl; carboxyaryl; C_{1-7} alkoxy; C_{3-10} cycloalkoxy; aryloxy; arylalkyloxy; oxyheterocyclic; heterocyclic-substituted alkyloxy; thio C_{1-7} alkyl; thio C_{3-10} cycloalkyl; thioaryl; thioheterocyclic; arylalkylthio; heterocyclic-substituted alkylthio; hydroxylamino; mercapto-amino; acylamino; thio-acylamino;

alkoxyamino; thioalkylamino; acetal; thio-acetal; carboxylic acid; carboxylic acid esters, thioesters, halides, anhydrides, amides and thioamides; thiocarboxylic acid; thiocarboxylic acid esters, thioesters, halides, anhydrides, amides and thioamides; hydroxyl; sulfhydryl; nitro; cyano; carbamoyl; thiocarbamoyl, ureido; thio-ureido; alkylamino; cycloalkylamino; alkenylamino; cycloalkenylamino; alkynylamino; arylamino; arylalkylamino; hydroxyalkylamino; mercaptoalkylamino; heterocyclic amino; heterocyclic-substituted alkylamino; oximino; alkyloximino; hydrazino; alkylhydrazino; phenylhydrazino; cysteinyl acid, esters, thioesters, halides, anhydrides, amides and thioamides thereof; aryl being optionally substituted with one or more substituents selected from the group consisting of halogen, C_{1-7} alkyl, C_{1-7} alkoxy, C_{2-7} alkenyl, C_{2-7} alkynyl, halo C_{1-7} alkyl, nitro, hydroxyl, sulfhydryl, amino, C_{3-10} cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, carbamoyl, thiocarbamoyl, ureido, thio-ureido, sulfonamido, hydroxylamino, alkoxyamino, mercaptoamino, thioalkylamino, acylamino, thioacyl-amino, cyano, carboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, thiocarboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino and phenylhydrazino; optionally substituted heterocyclic radicals; aromatic or heterocyclic substituents substituted with an aliphatic spacer between the pteridine ring and the aromatic or heterocyclic substituent, whereby said aliphatic spacer is a branched or straight, saturated or unsaturated aliphatic chain of 1 to 4 carbon atoms which may contain one or more functions, atoms or radicals selected from the group consisting of carbonyl (oxo), thiocarbonyl, alcohol (hydroxyl), thiol, ether, thio-ether, acetal, thio-acetal, amino, imino, oximino, alkyloximino, amino-acid, cyano, acylamino, thioacylamino, carbamoyl, thiocarbamoyl, ureido, thio-ureido, carboxylic acid or ester or thioester or halide or anhydride or amide, thiocarboxylic acid or ester or thioester or halide or anhydride or amide, nitro, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, hydroxylamino, mercaptoamino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenyl-amino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and halogen; branched or straight, saturated or unsaturated aliphatic chains of 1 to 7 carbon atoms optionally containing one or more functions selected from the group consisting of carbonyl (oxo), thiocarbonyl, alcohol (hydroxyl), thiol, ether, thio-ether, acetal, thio-acetal, amino, imino, oximino, alkyloximino, aminoacid, cyano, acylamino; thioacylamino; carbamoyl, thiocarbamoyl, ureido, thio-ureido, carboxylic acid ester or halide or anhydride or amide, thiocarboxylic acid or ester or thioester or halide or anhydride or amide, nitro, thio C_{1-7} alkyl, thio C_{3-10} cycloalkyl, hydroxylamino, mercaptoamino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynyl-amino, arylamino, arylalkylamino,

hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and halogen; and

- R_3 is selected from chloro and thiomethyl;

and/or a pharmaceutically acceptable addition salt thereof and/or a stereoisomer thereof and/or a mono- or a di-*N*-oxide thereof and/or a solvate and/or a dihydro- or tetrahydropteridine derivative thereof.

When applicable, and depending upon the specific substituents being present, not only the novel pteridine having the general formula (I) but also some pteridine derivatives previously known in the art without any indication of biological activity, i.e. all of the pteridines having the general formula (II) according to this invention, may be in the form of a pharmaceutically acceptable salt. The latter include any therapeutically active non-toxic addition salt which compounds having the general formula (II) are able to form with a salt-forming agent. Such addition salts may conveniently be obtained by treating the pteridine derivatives of the invention with an appropriate salt-forming acid or base. For instance, pteridine derivatives having basic properties may be converted into the corresponding therapeutically active, non-toxic acid addition salt form by treating the free base form with a suitable amount of an appropiate acid following conventional procedures. Examples of such appropriate salt-forming acids include, for instance, inorganic acids resulting in forming salts such as but not limited to hydrohalides (e.g.hydrochloride and hydrobromide), sulfate, nitrate, phosphate, diphosphate, carbonate, bicarbonate, and the like; and organic monocarboxylic or dicarboxylic acids resulting in forming salts such as, for example, acetate, propanoate, hydroxyacetate, 2-hydroxypropanoate, 2-oxopropanoate, lactate, pyruvate, oxalate, malonate, succinate, maleate, fumarate, malate, tartrate, citrate, methanesulfonate, ethanesulfonate, benzoate, 2-hydroxybenzoate, 4-amino-2-hydroxybenzoate, benzene-sulfonate, p-toluenesulfonate, salicylate, p-aminosalicylate, pamoate, bitartrate, camphorsulfonate, edetate, 1,2-ethanedisulfonate, fumarate, glucoheptonate, gluconate, glutamate, hexylresorcinate, hydroxynaphtoate, hydroxyethanesulfonate, mandelate, methylsulfate, pantothenate, stearate, as well as salts derived from ethanedioic, propanedioic, butanedioic, (Z)-2-butenedioic, (E)2-butenedioic, 2-hydroxybutanedioic, 2,3-dihydroxy-butanedioic, 2-hydroxy-1,2,3-propanetricarboxylic and cyclohexanesulfamic acids and the like.

Pteridine derivatives having acidic properties may be converted in a similar manner into the corresponding therapeutically active, non-toxic base addition salt form. Examples of appropriate salt-forming bases include, for instance, inorganic bases like metallic hydroxides such as but not limited to those of alkali and alkaline-earth metals like calcium, lithium, magnesium, potassium and sodium, or zinc, resulting in the corresponding metal salt; organic bases such as but not limited to ammonia, alkylamines, benzathine, hydrabamine, arginine, lysine, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylene-diamine, N-methylglucamine, procaine and the like.

Reaction conditions for treating the pteridine derivatives (II) of this invention with an appropriate salt-forming acid or base are similar to standard conditions involving the same

acid or base but different organic compounds with basic or acidic properties, respectively. Preferably, in view of its use in a pharmaceutical composition or in the manufacture of medicament for treating specific diseases, the pharmaceutically acceptable salt will be designed, i.e. the salt-forming acid or base will be selected so as to impart greater water-solubility, lower toxicity, greater stability and/or slower dissolution rate to the pteridine derivative of this invention.

The present invention further provides the use of a pteridine derivative represented by the general formula (II), or a pharmaceutically acceptable salt or a solvate thereof, as a biologically-active ingredient, i.e. an active principle, especially as a medicine or a diagnostic agent or for the manufacture of a medicament or a diagnostic kit. In particular the said medicament may be for the prevention or treatment of a pathologic condition selected from the group consisting of:

- immune disorders, in particular organ and cells transplant rejections, and autoimmune disorders,
- cardiovascular disorders,
- allergic conditions,
- disorders of the central nervous system, and
- cell proliferative disorders.

The pathologic conditions and disorders concerned by the said use, and the corresponding methods of prevention or treatment, are detailed hereinbelow. Any of the uses mentioned with respect to the present invention may be restricted to a non-medical use (e.g. in a cosmetic composition), a non-therapeutic use, a non-diagnostic use, a non-human use (e.g. in a veterinary composition), or exclusively an *in-vitro* use, or a use with cells remote from an animal.

The invention further relates to a pharmaceutical composition comprising:

(a) one or more pteridine represented by the general formula (II), and

(b) one or more pharmaceutically acceptable carriers.

In a third embodiment, this invention provides combinations, preferably synergistic combinations, of one or more pteridine represented by the general formula (II) with one or more biologically-active drugs being preferably selected from the group consisting of immunosuppressant and/or immunomodulator drugs, antineoplastic drugs, anti-histamines, inhibitors of allergy-causative agents (anti-allergic drugs) and antiviral agents. As is conventional in the art, the evaluation of a synergistic effect in a drug combination may be made by analyzing the quantification of the interactions between individual drugs, using the median effect principle described by Chou et al. in *Adv. Enzyme Reg.* (1984) 22:27. Briefly, this principle states that interactions (synergism, additivity, antagonism) between two drugs can be quantified using the combination index (hereinafter referred as CI) defined by the following equation:

$$CI_x = \frac{ED_x^{1c}}{ED_x^{1a}} + \frac{ED_x^{2c}}{ED_x^{2a}}$$

wherein ED_x is the dose of the first or respectively second drug used alone (1a, 2a), or in combination with the second or respectively first drug (1c, 2c), which is needed to produce a given effect. The said first and second drug have synergistic or additive or antagonistic effects depending upon CI < 1, CI = 1, or CI > 1, respectively. As will be explained in more detail herein-below, this principle may be applied to a number of desirable effects such as, but not limited to, an activity against transplant rejection, an activity against immunosuppression or immunomodulation, an activity against allergy or an activity against cell proliferation.

For instance the present invention relates to a pharmaceutical composition or combined preparation having synergistic effects against immuno-suppression or immunomodulation and containing:

(a) one or more immunosuppressant and/or immunomodulator drugs, and

(b) at least one pteridine derivative represented by the general formula (II), and

(c) optionally one or more pharmaceutical excipients or pharmaceutically acceptable carriers,

for simultaneous, separate or sequential use in the treatment or prevention of autoimmune disorders and/or in transplant-rejections.

Suitable immunosuppressant drugs for inclusion in the synergistic compositions or combined preparations of this invention belong to a well known therapeutic class. They are preferably selected from the group consisting of cyclosporin A, substituted xanthines (e.g. methylxanthines such as pentoxyfylline), daltroban, sirolimus, tacrolimus, rapamycin (and derivatives thereof such as defined below), leflunomide (or its main active metabolite A771726, or analogs thereof called malononitrilamides), mycophenolic acid and salts thereof (including the sodium salt marketed under the trade name Mofetil®), adrenocortical steroids, azathioprine, brequinar, gusperimus, 6-mercaptopurine, mizoribine, chloroquine, hydroxychloroquine and monoclonal antibodies with immunosuppressive properties (e.g. etanercept, infliximab or kineret). Adrenocortical steroids within the meaning of this invention mainly include glucocorticoids such as but not limited to ciprocinonide, desoxycorticisterone, fludrocortisone, flumoxonide, hydrocortisone, naflocort, procinonide, timobesone, tipredane, dexamethasone, methylprednisolone, methotrexate, prednisone, prednisolone, triamcinolone and pharmaceutically acceptable salts thereof. Rapamycin derivatives as referred herein include O-alkylated derivatives, particularly 9-deoxorapamycins, 26-dihydrorapamycins, 40-O-substituted rapamycins and 28,40-O,O-disubstituted rapamycins (as disclosed in U.S. Patent No. 5,665,772) such as 40-O-(2-hydroxy) ethyl rapamycin – also known as SDZ-RAD -, pegylated rapamycin (as disclosed in U.S. Patent No. 5,780,462), ethers of 7-desmethylrapamycin (as disclosed in U.S. Patent No. 6,440,991) and polyethylene glycol esters of SDZ-RAD (as disclosed in U.S. Patent No. 6,331,547).

Suitable immunomodulator drugs for inclusion into the synergistic immunomodulating pharmaceutical compositions or combined preparations of this invention are preferably

selected from the group consisting of acemannan, amiprilose, bucillamine, dimepranol, ditiocarb sodium, imiquimod, Inosine Pranobex, interferon-β, interferon-γ, lentinan, levamisole, lisophylline, pidotimod, romurtide, platonin, procodazole, propagermanium, thymomodulin, thymopentin and ubenimex.

Synergistic activity of the pharmaceutical compositions or combined preparations of this invention against immunosuppression or immuno-modulation may be readily determined by means of one or more lymphocyte activation tests. Usually activation is measured via lymphocyte proliferation. Inhibition of proliferation thus always means immunosuppression under the experimental conditions applied. There exist different stimuli for lymphocyte activation, in particular:

a) co-culture of lymphocytes of different species (mixed lymphocyte reaction, hereinafter referred as MLR) in a so-called mixed lymphocyte culture test: lymphocytes expressing different minor and major antigens of the HLA-DR type (= alloantigens) activate each other non-specifically;

b) a CD3 assay wherein there is an activation of the T-lymphocytes via an exogenously added antibody (OKT3). This antibody reacts against a CD3 molecule located on the lymphocyte membrane which has a co-stimulatory function. Interaction between OKT3 and CD3 results in T-cell activation which proceeds via the Ca^2+/calmodulin/calcineurin system and can be inhibited e.g. by cyclosporin A (hereinafter referred as CyA);

c) a CD28 assay wherein specific activation of the T-lymphocyte proceeds via an exogenously added antibody against a CD28 molecule which is also located on the lymphocyte membrane and delivers strong co-stimulatory signals. This activation is Ca^2+-independent and thus cannot be inhibited by CyA.

Determination of the immunosuppressing or immunomodulating activity of the pteridine derivatives of this invention, as well as synergistic combinations comprising them, is preferably based on the determination of one or more, preferably at least three lymphocyte activation *in vitro* tests, more preferably including at least one of the MLR test, CD3 assay and CD28 assay referred above. Preferably the lymphocyte activation *in vitro* tests used include at least two assays for two different clusters of differentiation preferably belonging to the same general type of such clusters and more preferably belonging to type I transmembrane proteins. Optionally the determination of the immuno-suppressing or immunomodulating activity may be performed on the basis of other lymphocyte activation *in vitro* tests, for instance by performing a TNF-α assay or an IL-1 assay or an IL-6 assay or an IL-10 assay or an IL-12 assay or an assay for a cluster of differentiation belonging to a further general type of such clusters and more preferably belonging to type II transmembrane proteins such as, but not limited to, CD69, CD 71 or CD134.

The synergistic effect may be evaluated by the median effect analysis method described herein-before. Such tests may for instance, according to standard practice in the art, involve the use of equiment, such as flow cytometer, being able to separate and sort a

number of cell subcategories at the end of the analysis, before these purified batches can be analysed further.

Synergistic activity of the pharmaceutical compositions of this invention in the prevention or treatment of transplant rejection may be readily determined by means of one or more leukocyte activation tests performed in a Whole Blood Assay (hereinafter referred as WBA) described for instance by Lin et al. in *Transplantation* (1997) 63:1734-1738. WBA used herein is a lymphoproliferation assay performed *in vitro* using lymphocytes present in the whole blood, taken from animals that were previously given the pteridine derivative, and optionally the other immunosuppressant drug, *in vivo* (more details are given in example 118). Hence this assay reflects the *in vivo* effect of substances as assessed by an *in vitro* read-out assay. The synergistic effect may be evaluated by the median effect analysis method described herein-before. Various organ transplantation models in animals are also available *in vivo*, which are strongly influenced by different immunogenicities, depending on the donor and recipient species used and depending on the nature of the transplanted organ. The survival time of transplanted organs can thus be used to measure the suppression of the immune response.

The pharmaceutical composition or combined preparation with synergistic activity against immunosuppression or immunomodulation according to this invention may contain the pteridine derivative of formula (II) over a broad content range depending on the contemplated use and the expected effect of the preparation. Generally, the pteridine derivative content of the combined preparation is within the range of 0.1 to 99.9% by weight, preferably from 1 to 99% by weight, more preferably from 5 to 95% by weight.

The invention further relates to a composition or combined preparation having synergistic effects against cell proliferation and containing:

(a) one or more antineoplastic drugs, and

(b) at least one pteridine deivative represented by the general formula (II), and

(c) optionally one or more pharmaceutical excipients or pharmaceutically acceptable carriers, for simultaneous, separate or sequential use in the treatment or prevention of cell proliferative disorders.

Suitable antineoplastic drugs for inclusion into the synergistic antiproliferative pharmaceutical compositions or combined preparations of this invention are preferably selected from the group consisting of alkaloids, alkylating agents (including but not limited to alkyl sulfonates, aziridines, ethylenimines, methylmelamines, nitrogen mustards and nitrosoureas), antibiotics, antimetabolites (including but not limited to folic acid analogs, purine analogs and pyrimidine analogs), enzymes, interferon and platinum complexes. More specific examples include acivicin; aclarubicin; acodazole; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene; bisnafide; bizelesin; bleomycin; brequinar; bropirimine;

busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin; decitabine; dexormaplatin; dezaguanine; diaziquone; docetaxel; doxorubicin; droloxifene; dromostanolone; duazomycin; edatrexate; eflomithine; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin; erbulozole; esorubicin; estramustine; etanidazole; ethiodized oil I 131; etoposide; etoprine; fadrozole; fazarabine; fenretinide; floxuridine; fludarabine; fluorouracil; flurocitabine; fosquidone; fostriecin; gemcitabine; Gold 198; hydroxyurea; idarubicin; ifosfamide; ilmofosine; interferon α-2a; interferon α-2b; interferon α-n1; interferon α-n3; interferon β-1a; interferon γ-1b; iproplatin; irinotecan; lanreotide; letrozole; leuprolide; liarozole; lometrexol; lomustine; losoxantrone; masoprocol; maytansine; mechlorethamine; megestrol; melengestrol; melphalan; menogaril; mercaptopurine; methotrexate; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone; mycophenolic acid; nocodazole; nogala-mycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin; perfosfamide; pipobroman; piposulfan; piroxantrone; plicamycin; plomestane; porfimer; porfiromycin; prednimustine; procarbazine; puromycin; pyrazofurin; riboprine; rogletimide; safingol; semustine; simtrazene; sparfosate; sparsomycin; spirogermanium; spiromustine; spiroplatin; streptonigrin; streptozocin; strontium 89 chloride; sulofenur; talisomycin; taxane; taxoid; tecogalan; tegafur; teloxantrone; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; topotecan; toremifene; trestolone; triciribine; trimetrexate; triptorelin; tubulozole; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine; vincristine; vindesine; vinepidine; vinglycinate; vinleurosine; vinorelbine; vinrosidine; vinzolidine; vorozole; zeniplatin; zinostatin; zorubicin; and their pharmaceutically acceptable salts.

Other suitable anti-neoplastic compounds include 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; anti-androgens such as, but not limited to, benorterone, cioteronel, cyproterone, delmadinone, oxendolone, topterone, zanoterone and their pharmaceutically acceptable salts; anti-estrogens such as, but not limited to, clometherone; delmadinone; nafoxidine; nitromifene; raloxifene; tamoxifen; toremifene; trioxifene and their pharmaceutically acceptable salts; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; β-lactam derivatives; β-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine;

budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors; castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; clomifene and analogues thereof; clotrimazole; collismycin A and B; combretastatin and analogues thereof; conagenin; crambescidin 816; cryptophycin and derivatives thereof; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine; cytolytic factor; cytostatin; dacliximab; dehydrodidemnin B; deslorelin; dexifosfamide; dexrazoxane; dexverapamil; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol; dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; elemene; emitefur; epristeride; estrogen agonists and antagonists; exemestane; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fluorodaunorunicin; forfenimex; formestane; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idoxifene; idramantone; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; iobenguane; iododoxorubicin; ipomeanol; irinotecan; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N; leinamycin; lenograstim; lentinan; leptolstatin; leukemia inhibiting factor; leuprorelin; levamisole; liarozole; lissoclinamide; lobaplatin; lombricine; lonidamine; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitors; mifepristone; miltefosine; mirimostim; mitoguazone; mitolactol; mitonafide; mitotoxin fibroblast growth factor-saporin; mofarotene; molgramostim; human chorionic gonadotrophin monoclonal antibody; mopidamol; mycaperoxide B; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone; pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; octreotide; okicenone; onapristone; ondansetron; ondansetron; oracin; osaterone; oxaliplatin; oxaunomycin; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; peldesine; pentosan; pentostatin; pentrozole; perflubron; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine; pirarubicin; piritrexim; placetin A and B; plasminogen activator inhibitor; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein kinase C inhibitors; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitors; retelliptine; rhenium 186 etidronate; rhizoxin; retinamide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; saintopin; sarcophytol A; sargramostim; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol;

somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; splenopentin; spongistatin 1; squalamine; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; suradista; suramin; swainsonine; tallimustine; tamoxifen; tauromustine; tazarotene; tecogalan; tellurapyrylium; telomerase inhibitors; temozolomide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; titanocene; topsentin; tretinoin; triacetyluridine; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; variolin B; velaresol; veramine; verdins; verteporfin; vinxaltine; vitaxin; zanoterone; zilascorb; and their pharmaceutically acceptable salts.

Synergistic activity of the pharmaceutical compositions or combined preparations of this invention against cell proliferation may be readily determined by means of one or more tests such as, but not limited to, the measurement of the radioactivity resulting from the incorporation of ^{3}H-thymidine in culture of tumor cell lines. For instance, different tumor cell lines are selected in order to evaluate the anti-tumor effects of the test compounds, such as but not limited to:

- RPMI1788: human Peripheral Blood Leucocytes (PBL) Caucasian tumor line,
- Jurkat: human acute T cell leukemia,
- EL4: C57Bl/6 mouse lymphoma, or
- THP-1: human monocyte tumor line.

Depending on the selected tumor cell line, different culture media may be used, such as for example:

- for RPMI1788 and THP-1: RPMI-1640 + 10% FCS + 1% NEAA + 1% sodium pyruvate + $5x10^{-5}$ mercapto-ethanol + antibiotics (G-418 0.45 µg/ml).
- for Jurkat and EL4: RPMI-1640 + 10% FCS + antibiotics (G-418 0.45 µg/ml).

In a specific embodiment of the synergy determination test, the tumor cell lines are harvested and a suspension of $0.27x10^{6}$ cells/ml in whole medium is prepared. The suspensions (150 µl) are added to a microtiter plate in triplicate. Either complete medium (controls) or the test compounds at the test concentrations (50 µl) are added to the cell suspension in the microtiter plate. The cells are incubated at 37°C under 5% CO_2 for about 16 hours. ^{3}H-thymidine is added, and the cells incubated for another 8 hours. The cells are harvested and radioactivity is measured in counts per minute (CPM) in a ß-counter. The ^{3}H-thymidine cell content, and thus the measured radioactivity, is proportional to the proliferation of the cell lines. The synergistic effect is evaluated by the median effect analysis method as disclosed herein-before.

The pharmaceutical composition or combined preparation with synergistic activity against cell proliferation according to this invention may contain the pteridine compound of formula (II) over a broad content range depending on the contemplated use and the expected effect of the preparation. Generally, the pteridine content of the combined preparation is within the range

of 0.1 to 99.9% by weight, preferably from 1 to 99% by weight, more preferably from 5 to 95% by weight.

The invention further relates to a pharmaceutical composition or combined preparation having synergistic effects against a viral infection and containing:

(a) one or more anti-viral agents, and

(b) at least one pteridine derivative represented by the general formula (II), and

(c) optionally one or more pharmaceutical excipients or pharmaceutically acceptable carriers, for simultaneous, separate or sequential use in the treatment or prevention of a viral infection.

Suitable anti-viral agents for inclusion into the synergistic antiviral compositions or combined preparations of this invention include, for instance, retroviral enzyme inhibitors belonging to categories well known in the art, such as HIV-1 IN inhibitors, nucleoside reverse transcriptase inhibitors (e.g. zidovudine, lamivudine, didanosine, stavudine, zalcitabine and the like), non-nucleoside reverse transcriptase inhibitors (e.g. nevirapine, delavirdine and the like), other reverse transcriptase inhibitors (e.g. foscamet sodium and the like), and HIV-1 protease inhibitors (e.g. saquinavir, ritonavir, indinavir, nelfinavir and the like). Other suitable antiviral agents include for instance acemannan, acyclovir, adefovir, alovudine, alvircept, amantadine, aranotin, arildone, atevirdine, avridine, cidofovir, cipamfylline, cytarabine, desciclovir, disoxaril, edoxudine, enviradene, enviroxime, famciclovir, famotine, fiacitabine, fialuridine, floxuridine, fosarilate, fosfonet, ganciclovir, idoxuridine, kethoxal, lobucavir, memotine, methisazone, penciclovir, pirodavir, somantadine, sorivudine, tilorone, trifluridine, valaciclovir, vidarabine, viroxime, zinviroxime, moroxydine, podophyllotoxin, ribavirine, rimantadine, stallimycine, statolon, tromantadine and xenazoic acid, and their pharmaceutically acceptable salts.

Especially relevant to this aspect of the invention is the inhibition of the replication of viruses selected from the group consisting of picorna-, toga-, bunya, orthomyxo-, paramyxo-, rhabdo-, retro-, arena-, hepatitis B-, hepatitis C-, hepatitis D-, adeno-, vaccinia-, papilloma-, herpes-, corona-, varicella- and zoster-virus, in particular human immunodeficiency virus (HIV).Synergistic activity of the pharmaceutical compositions or combined preparations of this invention against viral infection may be readily determined by means of one or more tests such as, but not limited to, the isobologram method, as previously described by Elion et al. in *J. Biol. Chem.* (1954) 208:477-488 and by Baba et al. in *Antimicrob. Agents Chemother.* (1984) 25:515-517, using EC_{50} for calculating the fractional inhibitory concentration (hereinafter referred as FIC). When the minimum FIC index corresponding to the FIC of combined compounds (e.g., FIC_x + FIC_y) is equal to 1.0, the combination is said to be additive; when it is beween 1.0 and 0.5, the combination is defined as subsynergistic, and when it is lower than 0.5, the combination is by defined as synergistic. When the minimum FIC index is between 1.0 and 2.0, the combination is defined as subantagonistic and, when it is higher than 2.0, the combination is defined as antagonistic.

The pharmaceutical composition or combined preparation with synergistic activity against viral infection according to this invention may contain the pteridine compound of formula (II) over a broad content range depending on the contemplated use and the expected effect of the preparation. Generally, the pteridine content of the combined preparation is within the range of 0.1 to 99.9% by weight, preferably from 1 to 99% by weight, more preferably from 5 to 95% by weight.

The pharmaceutical compositions and combined preparations according to this invention may be administered orally or in any other suitable fashion. Oral administration is preferred and the preparation may have the form of a tablet, aqueous dispersion, dispersable powder or granule, emulsion, hard or soft capsule, syrup, elixir or gel. The dosing forms may be prepared using any method known in the art for manufacturing these pharmaceutical compositions and may comprise as additives sweeteners, flavoring agents, coloring agents, preservatives and the like. Carrier materials and excipients are detailed hereinbelow and may include, *inter alia*, calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, binding agents and the like. The pharmaceutical composition or combined preparation of this invention may be included in a gelatin capsule mixed with any inert solid diluent or carrier material, or has the form of a soft gelatin capsule, in which the ingredient is mixed with a water or oil medium. Aqueous dispersions may comprise the biologically active composition or combined preparation in combination with a suspending agent, dispersing agent or wetting agent. Oil dispersions may comprise suspending agents such as a vegetable oil. Rectal administration is also applicable, for instance in the form of suppositories or gels. Injection (e.g. intramuscularly or intraperiteneously) is also applicable as a mode of administration, for instance in the form of injectable solutions or dispersions, depending upon the disorder to be treated and the condition of the patient.

Auto-immune disorders to be prevented or treated by the pharma-ceutical compositions or combined preparations of this invention include both systemic auto-immune diseases such as but not limited to lupus erythematosus, psoriasis, vasculitis, polymyositis, scleroderma, multiple sclerosis, ankylosing spondilytis, rheumatoid arthritis and Sjögren syndrome; auto-immune endocrine disorders such as thyroiditis; and organ-specific auto-immune diseases such as but not limited to Addison disease, hemolytic or pernicious anemia, Goodpasture syndrome, Graves disease, idiopathic thrombocytopenic purpura, insulin-dependent diabetes mellitus, juvenile diabetes, uveitis, Crohn's disease, ulcerative colitis, pemphigus, atopic dermatitis, autoimmune hepatitis, primary biliary cirrhosis, autoimmune pneumonitis, autoimmune carditis, myasthenia gravis, glomerulonephritis and spontaneous infertility.

Transplant rejections to be prevented or treated by the pharmaceutical compositions or combined preparations of this invention include the rejection of transplanted or grafted organs or cells (both allografts and xenografts), such as but not limited to host versus graft reaction

disease. The term " organ" as used herein means all organs or parts of organs in mammals, in particular humans, such as but not limited to kidney, lung, bone marrow, hair, cornea, eye (vitreous), heart, heart valve, liver, pancreas, blood vessel, skin, muscle, bone, intestine or stomach. " Rejection " as used herein mean all reactions of the recipient body or of the transplanted organ which in the end lead to cell or tissue death in the transplanted organ or adversely affect the functional ability and viability of the transplanted organ or the recipient. In particular, this means acute and chronic rejection reactions. Also included in this invention is preventing or treating the rejection of cell transplants and xenotransplantation. The major hurdle for xenotransplantation is that even before the T lymphocytes, responsible for the rejection of allografts, are activated, the innate immune system, especially T-independent B lymphocytes and macrophages are activated. This provokes two types of severe and early acute rejection called hyper-acute rejection and vascular rejection, respectively. The present invention addresses the problem that conventional immunosuppressant drugs like cyclosporin A are ineffective in xenotransplantation. The ability of the compounds of this invention to suppress T-independent xeno-antibody production as well as macrophage activation may be evaluated in the ability to prevent xenograft rejection in athymic, T-deficient mice receiving xenogenic hamster-heart grafts.

Cell proliferative disorders to be prevented or treated by the pharmaceutical compositions or combined preparations of this invention include any kind of tumor progression or invasion or metastasis inhibition of a cancer, preferably one selected from the group consisting of lung cancer, leukaemia, ovarian cancer, sarcoma, Kaposi's sarcoma, meningioma, colon cancer, lymp node tumor, glioblastoma multiforme, prostate cancer or skin carcinose.

CNS disorders to be prevented or treated by the pharmaceutical compositions of this invention include cognitive pathologies such as dementia, cerebral ischemia, trauma, epilepsy, schizophrenia, chronic pain and neurologic disorders such as but not limited to depression, social phobia and obsessive compulsive disorders.

Cardiovascular disorders to be prevented or treated by the pharmaceutical compositions of this invention include ischemic disorders, infarct or reperfusion damage, atherosclerosis and stroke.

Allergic conditions to be prevented or treated by the pharmaceutical compositions of this invention include those caused by the pollen of graminae, the presence of pets, as well as more severe forms, such as asthma, characterized by inflammation of airways and bronchospasm. Without wishing to be bound by theory, the antiallergic effect of the compounds of the invention may be related to their suppression of certain B-cell activation pathways, which can lead to the suppression of IgE release. It may also be related to their properties of inhibiting certain Th2 cytokines, such as IL-5, IL-13 or IL-10, involved in asthma.

The term " pharmaceutically acceptable carrier or excipient " as used herein in relation to pharmaceutical compositions and combined preparations means any material or substance with which the active principle, i.e. the pteridine derivative of formula (II), and optionally the immunosuppressant or immunomodulator or antineoplastic drug or antiviral agent, may be

formulated in order to facilitate its application or dissemination to the locus to be treated, for instance by dissolving, dispersing or diffusing the said composition, and/or to facilitate its storage, transport or handling without impairing its effectiveness. The pharmaceutically acceptable carrier may be a solid or a liquid or a gas which has been compressed to form a liquid, i.e. the compositions of this invention can suitably be used as concentrates, emulsions, solutions, granulates, dusts, sprays, aerosols, pellets or powders.

Suitable pharmaceutical carriers for use in the said pharmaceutical compositions and their formulation are well known to those skilled in the art. There is no particular restriction to their selection within the present invention although, due to the usually low or very low water-solubility of the pteridine derivatives of this invention, special attention will be paid to the selection of suitable carrier combinations that can assist in properly formulating them in view of the expected time release profile. Suitable pharmaceutical carriers include additives such as wetting agents, dispersing agents, stickers, adhesives, emulsifying or surface-active agents, thickening agents, complexing agents, gelling agents, solvents, coatings, antibacterial and antifungal agents (for example phenol, sorbic acid, chlorobutanol), isotonic agents (such as sugars or sodium chloride) and the like, provided the same are consistent with pharmaceutical practice, i.e. carriers and additives which do not create permanent damage to mammals. The pharmaceutical compositions of the present invention may be prepared in any known manner, for instance by homogeneously mixing, dissolving, spray-drying, coating and/or grinding the active ingredients, in a one-step or a multi-steps procedure, with the selected carrier material and, where appropriate, the other additives such as surface-active agents. may also be prepared by micronisation, for instance in view to obtain them in the form of microspheres usually having a diameter of about 1 to 10 µm, namely for the manufacture of microcapsules for controlled or sustained release of the biologically active ingredient(s).

Suitable surface-active agents to be used in the pharmaceutical compositions of the present invention are non-ionic, cationic and/or anionic materials having good emulsifying, dispersing and/or wetting properties. Suitable anionic surfactants include both water-soluble soaps and water-soluble synthetic surface-active agents. Suitable soaps are alkaline or alkaline-earth metal salts, unsubstituted or substituted ammonium salts of higher fatty acids (C_{10}-C_{22}), e.g. the sodium or potassium salts of oleic or stearic acid, or of natural fatty acid mixtures obtainable form coconut oil or tallow oil. Synthetic surfactants include sodium or calcium salts of polyacrylic acids; fatty sulphonates and sulphates; sulphonated benzimidazole derivatives and alkylarylsulphonates. Fatty sulphonates or sulphates are usually in the form of alkaline or alkaline-earth metal salts, unsubstituted ammonium salts or ammonium salts substituted with an alkyl or acyl radical having from 8 to 22 carbon atoms, e.g. the sodium or calcium salt of lignosulphonic acid or dodecylsulphonic acid or a mixture of fatty alcohol sulphates obtained from natural fatty acids, alkaline or alkaline-earth metal salts of sulphuric or sulphonic acid esters (such as sodium lauryl sulphate) and sulphonic acids of fatty alcohol/ethylene oxide adducts. Suitable sulphonated benzimidazole derivatives

preferably contain 8 to 22 carbon atoms. Examples of alkylarylsulphonates are the sodium, calcium or alcanolamine salts of dodecylbenzene sulphonic acid or dibutyl-naphtalenesulphonic acid or a naphtalene-sulphonic acid/formaldehyde condensation product. Also suitable are the corresponding phosphates, e.g. salts of phosphoric acid ester and an adduct of p-nonylphenol with ethylene and/or propylene oxide, or phospholipids. Suitable phospholipids for this purpose are the natural (originating from animal or plant cells) or synthetic phospholipids of the cephalin or lecithin type such as e.g. phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerine, lysolecithin, cardiolipin, dioctanyl-phosphatidylcholine, dipalmitoylphoshatidylcholine and their mixtures.

Suitable non-ionic surfactants include polyethoxylated and polypropoxylated derivatives of alkylphenols, fatty alcohols, fatty acids, aliphatic amines or amides containing at least 12 carbon atoms in the molecule, alkylarenesulphonates and dialkylsulphosuccinates, such as polyglycol ether derivatives of aliphatic and cycloaliphatic alcohols, saturated and unsaturated fatty acids and alkylphenols, said derivatives preferably containing 3 to 10 glycol ether groups and 8 to 20 carbon atoms in the (aliphatic) hydrocarbon moiety and 6 to 18 carbon atoms in the alkyl moiety of the alkylphenol. Further suitable non-ionic surfactants are water-soluble adducts of polyethylene oxide with poylypropylene glycol, ethylenediaminopolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain, which adducts contain 20 to 250 ethyleneglycol ether groups and/or 10 to 100 propyleneglycol ether groups. Such compounds usually contain from 1 to 5 ethyleneglycol units per propyleneglycol unit. Representative examples of non-ionic surfactants are nonylphenol-polyethoxyethanol, castor oil polyglycolic ethers, polypropylene/ polyethylene oxide adducts, tributylphenoxypolyethoxyethanol, polyethyleneglycol and octylphenoxypolyethoxyethanol. Fatty acid esters of polyethylene sorbitan (such as polyoxyethylene sorbitan trioleate), glycerol, sorbitan, sucrose and pentaerythritol are also suitable non-ionic surfactants.

Suitable cationic surfactants include quaternary ammonium salts, preferably halides, having 4 hydrocarbon radicals optionally substituted with halo, phenyl, substituted phenyl or hydroxy; for instance quaternary ammonium salts containing as N-substituent at least one C_8-C_{22} alkyl radical (e.g. cetyl, lauryl, palmityl, myristyl, oleyl and the like) and, as further substituents, unsubstituted or halogenated lower alkyl, benzyl and/or hydroxy-lower alkyl radicals.

A more detailed description of surface-active agents suitable for this purpose may be found for instance in "McCutcheon's Detergents and Emulsifiers Annual" (MC Publishing Crop., Ridgewood, New Jersey, 1981), "Tensid-Taschenbuch", 2nd ed. (Hanser Verlag, Vienna, 1981) and "Encyclopaedia of Surfactants (Chemical Publishing Co., New York, 1981).

Structure-forming, thickening or gel-forming agents may be included into the pharmaceutical compositions and combined preparations of the invention. Suitable such

agents are in particular highly dispersed silicic acid, such as the product commercially available under the trade name Aerosil; bentonites; tetraalkyl ammonium salts of montmorillonites (e.g., products commercially available under the trade name Bentone), wherein each of the alkyl groups may contain from 1 to 20 carbon atoms; cetostearyl alcohol and modified castor oil products (e.g. the product commercially available under the trade name Antisettle).

Gelling agents which may be included into the pharmaceutical compositions and combined preparations of the present invention include, but are not limited to, cellulose derivatives such as carboxymethylcellulose, cellulose acetate and the like; natural gums such as arabic gum, xanthum gum, tragacanth gum, guar gum and the like; gelatin; silicon dioxide; synthetic polymers such as carbomers, and mixtures thereof. Gelatin and modified celluloses represent a preferred class of gelling agents.

Other optional excipients which may be included in the pharmaceutical compositions and combined preparations of the present invention include additives such as magnesium oxide; azo dyes; organic and inorganic pigments such as titanium dioxide; UV-absorbers; stabilisers; odor masking agents; viscosity enhancers; antioxidants such as, for example, ascorbyl palmitate, sodium bisulfite, sodium metabisulfite and the like, and mixtures thereof; preservatives such as, for example, potassium sorbate, sodium benzoate, sorbic acid, propyl gallate, benzylalcohol, methyl paraben, propyl paraben and the like; sequestering agents such as ethylene-diamine tetraacetic acid; flavoring agents such as natural vanillin; buffers such as citric acid and acetic acid; extenders or bulking agents such as silicates, diatomaceous earth, magnesium oxide or aluminum oxide; densification agents such as magnesium salts; and mixtures thereof.

Additional ingredients may be included in order to control the duration of action of the biologically-active ingredient in the compositions and combined preparations of the invention. Control release compositions may thus be achieved by selecting appropriate polymer carriers such as for example polyesters, polyamino-acids, polyvinyl-pyrrolidone, ethylene-vinyl acetate copolymers, methylcellulose, carboxymethylcellulose, protamine sulfate and the like. The rate of drug release and duration of action may also be controlled by incorporating the active ingredient into particles, e.g. microcapsules, of a polymeric substance such as hydrogels, polylactic acid, hydroxymethyl-cellulose, polymethyl methacrylate and the other above-described polymers. Such methods include colloid drug delivery systems like liposomes, microspheres, microemulsions, nanoparticles, nanocapsules and so on. Depending on the route of administration, the pharmaceutical composition or combined preparation of the invention may also require protective coatings.

Pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation thereof. Typical carriers for this purpose therefore include biocompatible aqueous buffers, ethanol, glycerol, propylene

glycol, polyethylene glycol, complexing agents such as cyclodextrins and the like, and mixtures thereof.

Since, in the case of combined preparations including the pteridine derivative of this invention and an immunosuppressant or immunomodulator or antihistamine or antineoplastic drug or antiviral agent, both ingredients do not necessarily bring out their synergistic therapeutic effect directly at the same time in the patient to be treated, the said combined preparation may be in the form of a medical kit or package containing the two ingredients in separate but adjacent form. In the latter context, each ingredient may therefore be formulated in a way suitable for an administration route different from that of the other ingredient, e.g. one of them may be in the form of an oral or parenteral formulation whereas the other is in the form of an ampoule for intravenous injection or an aerosol.

The present invention further relates to a method for preventing or treating a disease selected from the group consisting of CNS disorders, cell proliferative disorders, allergic conditions, viral infections, immune and auto-immune disorders and transplant rejections in a subject or patient by administering to the patient in need thereof an effective amount of a pteridine derivative having the general formula (II), optionally together with an effective amount of another immunosuppressant or immunomodulator or antineoplastic drug or antiviral agent, or a pharmaceutical composition such as disclosed above in extensive details. The effective amount is usually in the range of 0.01 mg to 20 mg, preferably 0.1 mg to 5 mg, per day per kg bodyweight for humans. Depending upon the pathologic condition to be treated and the patient's condition, the said effective amount may be divided into several sub-units per day or may be administered at more than one day intervals. The patient to be treated may be any warm-blooded animal, preferably a human being, suffering from said pathologic condition.

The following examples are intended to illustrate and not to limit the scope of the present invention in all its aspects.

Example 1 - synthesis of 2,6-diamino-4-ethoxy-pyrimidine

To a solution of sodium (1.05 g) in ethanol (50 ml) was added 4-chloro-2,6-diaminopyrimidine (6 g, 41.4 mmoles). The resulting solution was heated in a reactor for 6 hours at 160 °C. The reaction mixture was cooled down and the precipitated sodium chloride was filtered off. The filtrate was concentrated and precipitated from ethanol (two times), affording the pure title compound as a white solid (4.53 g, 72% yield). The spectral data are identical to those described e.g. by W. Pfleiderer et al. in *Chem. Ber.* (1961) *94*, 12.

Example 2 - synthesis of 2,6-diamino-4-isopropoxy-pyrimidine

The same procedure as in example 1 was followed using isopropanol instead of ethanol. The filtrate was pure enough for further reaction without purification. The spectral data are identical to those described e.g. by W. Pfleiderer et al. in *Chem. Ber.* (1961) *94*, 12.

Example 3 - synthesis of 5-nitroso-2,6-diamino-4-ethoxy-pyrimidine

To a solution of the compound of example 51 (6.13 g, 39.8 mmoles) in 20 % aqueous acetic acid (57 ml) was added dropwise a solution of $NaNO_2$ (3.29 g) in water (13 ml) at 80 °C. A pink precipitate was formed and stirred at 80°C for an additional 2 hours. The reaction mixture was cooled down in the refrigerator overnight and the resulting precipitate was filtered off, yielding the title compound as a pink powder (4.98 g, yield 68 %). Spectral data are identical with those described e.g. by W. Pfleiderer et al. in *Chem. Ber.* (1961) *94*, 12.

Example 4 - synthesis of 5-nitroso-2,6-diamino-4-isopropoxy-pyrimidine

The same procedure was followed as in example 3 but starting from the compound of example 2. The product has identical spectral data to those described by W. Pfleiderer et al. (cited *supra*).

Example 5 - synthesis of 2,5,6-triamino-4-ethoxy-pyrimidine

To a suspension of the compound of example 3 (7.12 g, 38.9 mmoles) in water (150 ml) at 60 °C was added sodium dithionite (46.7 mmol, 8.12 g). Additional sodium dithionite was added till the pink colour completely dis-appeared and a yellow solution was formed. The solution was stirred at 60 °C for another 4 hours. Water was evaporated and the resulting residue was precipitated from a small amount of water, providing the title compound as a yellow powder (4.02 g, yield 61 %). Spectral data are identical with literature data (W. Pfleiderer et al. cited *supra*).

Example 6 - synthesis of 2,5,6-triamino-4-isopropoxy-pyrimidine

The procedure of example 5 was followed, however using the compound of example 4 as the starting material. The spectral data of the product obtained are identical with the literature data (W. Pfleiderer et al. cited *supra*).

Example 7 - synthesis of 2-amino-4-ethoxy-pteridin

To a solution of 2,5,6-triamino-4-ethoxy-pyrimidine (10.54 g, 62.37 mmoles) in ethanol (160 ml) was added glyoxal (40 % solution in water, 2.7 ml, 18.6 mmoles). The reaction mixture was refluxed for 4 hours. Some insoluble material was filtered off. The filtrate was concentrated *in vacuo* and the residue purified by flash chromatography (silica, using a CH_3OH/CH_2Cl_2 mixture (5:95) as the eluent), providing the pure title compound (7.34 g, yield: 62 %). The spectral data of the product are identical with the literature data (W. Pfleiderer et al. cited *supra*).

Example 8 - synthesis of 2-amino-4-isopropoxy-pteridin

The procedure of example 7 was repeated, however using isopropanol as the solvent instead of ethanol. The spectral data of the product obtained are identical with the literature data (W. Pfleiderer et al. cited *supra*).

Example 9 - synthesis of 2-amino-4-ethoxypteridine-N^8-oxide

To a cooled (0 °C) solution of the compound of example 7 (2.47 g, 12.9 mmoles) in trifluoroacetic acid (53 ml) was added dropwise 2.53 ml of a 35 % aqueous H_2O_2 solution. The reaction mixture was kept at 4 °C for two days in the refrigerator, whereby another 1.25 ml of the same H_2O_2 solution was added after 1 day. The solution was concentrated *in vacuo*. The residue was suspended in water and neutralized by the addition of a concentrated ammonia solution. Evaporation of the solvent *in vacuo* and purification of the residue by flash chromatography (silica, using a CH_3OH/CH_2Cl_2 mixture (6:94) as the eluent) provided the title compound as a yellow powder (861 mg, yield: 32 %). Mass spectrum data are as follows: *m/z* (%): 230 ($[M+Na]^+$, 30), 208 ($[M+H]^+$, 100), 180 [$(M+H\text{-ethene})^+$, 10].

Example 10 - synthesis of 2-amino-4-isopropoxypteridine-N^8-oxide

The procedure as described in example 9 was followed, however using the compound of example 61 as the starting material. Mass spectrum data are as follows: *m/z* (%): 222 ($[M+H]^+$, 100), 180 ($[M+H\text{-propene}]^+$, 60).

Example 11 - synthesis of 2-amino-6-chloro-4-ethoxypteridine

A suspension of the compound of example 9 (460 mg, 2.22 mmoles) in acetyl chloride (5.5 ml) was stirred at -40 °C. Trifluoroacetic acid (1.69 ml) was then added dropwise. The resulting solution was slowly warmed up to 0 °C and stirred for an additional 4 hours at 0 °C. Reaction was carefully quenched with ice, followed by neutralization with a concentrated ammonia solution (pH = 8). The aqueous phase was extracted with CH_2Cl_2 (five times). The combined organic layers were concentrated *in vacuo* and the residue was purified by flash chromatography (silica, using a CH_3OH/CH_2Cl_2 mixture (1:99) as the eluent), thus providing the title compound as a yellow powder (360 mg, yield: 72 %). This compound was further characterized as follows :

- mass spectrum: *m/z* (%): 226 ($[M+H]^+$, 100),
- ^{1}H-NMR (200 MHz, DMSO-d_6): δ 1.42 (3 H, t), 4.52 (2 H, q), 7.42 (2 H, d) and 8.85 (1 H, s) ppm,
- ^{13}C-NMR (50 MHz, DMSO-d_6): δ 14.19, 63.58, 121.74, 140.22, 150.99, 156.13, 161.98 and 165.97 ppm.

Example 12 - synthesis of 2-amino-6-chloro-4-isopropoxypteridine

The procedure as described in example 11 was followed, however starting from the compound of example 63. The mass spectrum data of the resulting compound are as follows: *m/z* (%): 240 ($[M+H]^+$, 55), 198 ($[M+H\text{-propene}]^+$, 100).

Examples 13 to 30 - synthesis of 2-amino-6-aryl-4-ethoxypteridines and 2-amino-6-heteroaryl-4-ethoxypteridines

The general procedure used for preparing 2-amino-6-aryl-4-ethoxy-pteridines is as follows: to a degassed solution of the compound of example 64 (50 mg, 0.22 mmole) in THF (5 ml) was added a degassed solution of sodium carbonate (5 ml of a 0.4 M solution in water), tetrakis(triphenyl-phosphine) palladium (0.013 mmole, 14 mg) and an arylboronic or (examples 72 and 73) heteroarylboronic acid (0.22 mmole). The solution was refluxed for 4 hours. Solvents were concentrated *in vacuo* and the residue was purified by flash chromatography (silica) with an appropriate CH_3OH/CH_2Cl_2 mixture (2:98 or 3:97) as the eluent (except for the compound of example 82, which was eluted with an acetone/CH_2Cl_2 (7:3) mixture). This procedure provided, with a yield ranging from 16 % to 60 % depending upon the aryl or heteroaryl group (from the arylboronic or heteroarylboronic acid) introduced at the 6-position of the pteridine ring, the following pure final compounds which were characterized by their mass spectrum MS and optionally by their ^{1}H-NMR (200 MHz, DMSO-d_6) spectrum:

- 2-amino-6-(p-methoxyphenyl)-4-ethoxy-pteridine (example 13): MS 298 ($[M+H]^+$, 100), 270 ($[M+H\text{-ethene}]^+$, 55) ;
- 2-amino-6-(o-methoxyphenyl)-4-ethoxy-pteridine (example 14): MS 298 ($[M+H]^+$, 100), 270 ($[M+H\text{-ethene}]^+$, 30) ;
- 2-amino-6-(m-methoxyphenyl)-4-ethoxy-pteridine (example 15) : MS 298 ($[M+H]^+$, 100), 270 ($[M+H\text{-ethene}]^+$, 35); ^{1}H-NMR: 1.46 (3 H, t), 3.85 (3 H, s), 4.58 (2 H, q), 7.06 (1 H, dd), 7.33 (2 H, br s), 7.46 (1 H, t), 7.68 (1 H, m) and 9.43 (1 H, s) ppm ;
- 2-amino-6-(3,4-difluorophenyl)-4-ethoxy-pteridine (example 16) : MS 304 ($[M+H]^+$,100), 270 ($[M+H\text{-ethene}]^+$, 35); ^{1}H-NMR: 1.45 (3 H, t), 4.57 (2 H, q), 7.42 (2 H, br s), 7.60 (1 H, q), 7.98 (1 H, d), 8.16 (1 H, t) and 9.42 (1 H, s) ppm ;
- 2-amino-6-(p-dimethylaminophenyl)-4-ethoxy-pteridine (example 17) : MS 311 ($[M+H]^+$,100), 283 ($[M+H\text{-ethene}]^+$, 35) ;
- 2-amino-6-(p-trifluoromethylphenyl)-4-ethoxy-pteridine (example 18) : MS 336 ($[M+H]^+$,100), 308 ($[M+H\text{-ethene}]^+$, 50) ;
- 2-amino-6-(2-thienyl)-4-ethoxy-pteridine (example 19): MS 274 ($[M+H]^+$, 100), 246 ($[M+H\text{-ethene}]^+$, 40) ;
- 2-amino-6-(3-thienyl)-4-ethoxy-pteridine (example 20): MS 274 ($[M+H]^+$, 100), 246 ($[M+H\text{-ethene}]^+$, 45) ;
- 2-amino-6-(3,4-dichlorophenyl)-4-ethoxy-pteridine (example 21): MS 337 ($[M+H]^+$,100); ^{1}H-NMR: 1.46 (3 H, t), 4.59 (2 H, q), 7.42 (2 H, br s), 7.81 (1H, d), 8.14 (1 H, dd), 8.37 (1 H, d) and 9.47 (1 H, s) ppm ;
- 2-amino-6-(p-cyanophenyl)-4-ethoxy-pteridine (example 22) : MS 293 ($[M+H]^+$,100), 265 ($[M+H\text{-ethene}]^+$, 65);
- 2-amino-6-(p-ethoxyphenyl)-4-ethoxy-pteridine (example 23): MS 312 ($[M+H]^+$, 100), 284 ($[M+H\text{-ethene}]^+$, 70) ;

- 2-amino-6-(p-fluorophenyl)-4-ethoxy-pteridine (example 24): MS 286 ($[M+H]^+$, 100), 258 ($[M+H\text{-ethene})^+$, 45) ;
- 2-amino-6-(p-ethylphenyl)-4-ethoxy-pteridine (example 25): MS 296 ($[M+H]^+$, 100), 268 ($[M+H\text{-ethene})^+$, 45) ;
- 2-amino-6-(p-acetylphenyl)-4-ethoxy-pteridine (example 26): MS 310 ($[M+H]^+$, 100), 282 ($[M+H\text{-ethene}]^+$, 60) ;
- 2-amino-6-(3-methyl-4-fluorophenyl)-4-ethoxy-pteridine (example 27): MS 300 ($[M+H]^+$, 100), 272 ($[M+H\text{-ethene}]^+$, 30) ;
- 2-amino-6-(p-thiomethylphenyl)-4-ethoxy-pteridine (example 28): MS 314 ($[M+H]^+$, 100), 286 ($[M+H\text{-ethene}]^+$, 35) ;
- 2-amino-6-(p-N,N-dimethylbenzamido)-4-ethoxy-pteridine (example 29) MS 338 ($[M+H]^+$, 100), 311 ($[M+H\text{-ethene}]^+$, 15) ; and
- 2-amino-6-(3,4-dimethoxyphenyl)-4-ethoxy-pteridine (example 30): MS 328 ($[M+H]^+$, 100), 300 ($[M+H\text{-ethene}]^+$, 40).

Examples 31 to 45 - synthesis of 2-amino-6-aryl-4-isopropoxypteridines and 2-amino-6-heteroaryl-4-isopropoxypteridines

The procedure as described in examples 13 to 30 was followed while using 2-amino-6-chloro-4-isopropoxypteridine as the starting material, except that longer reaction times were needed (refluxing overnight instead of 4 hours). This procedure provided, with a yield ranging from 10 % to 70 % depending upon the aryl or heteroaryl group introduced at the 6-position of the pteridine ring, the following pure final compounds which were characterized by their mass spectrum:

- 2-amino-6-(3-methyl-4-methoxyphenyl)-4-isopropoxypteridine (example 31) : MS 326 ($[M+H]^+$,100), 284 ($[M+H\text{-propene}]^+$, 30) ;
- 2-amino-6-(3,4-dimethylphenyl)-4-isopropoxypteridine (example 32) : MS 310 ($[M+H]^+$,100), 268 ($[M+H\text{-propene}]^+$, 60) ;
- 2-amino-6-(3-chloro-4-trifluoromethylphenyl)-4-isopropoxypteridine (example 33) : MS 384 ($[M+H]^+$, 20), 342 ($[M+H\text{-propene}]^+$, 50) ;
- 2-amino-6-(3-chloro-4-fluorophenyl)-4-isopropoxypteridine (example 34) : MS 334 ($[M+H]^+$, 20), 292 ($[M+H\text{-propene}]^+$, 50) ;
- 2-amino-6-(p-N,N-diethylbenzamido)-4-isopropoxypteridine (example 35) : MS 381 ($[M+H]^+$, 100) ;
- 2-amino-6-(p-trifluoromethylphenyl)-4-isopropoxypteridine (example 36): MS 350 ($[M+H]^+$, 100), 308 ($[M+H\text{-propene}]^+$, 30) ;
- 2-amino-6-(3,4-difluorophenyl)-4-isopropoxypteridine (example 37) : MS 318 ($[M+H]^+$, 100), 276 ($[M+H\text{-propene}]^+$, 50) ;
- 2-amino-6-(p-methoxyphenyl)-4-isopropoxypteridine (example 38) : MS 312 ($[M+H]^+$, 100), 270 ($[M+H\text{-propene}]^+$, 50) ;

- 2-amino-6-(p-ethoxyphenyl)-4-isopropoxypteridine (example 39): MS 326 ($[M+H]^+$, 55), 284 ($[M+H\text{-propene}]^+$, 100) ;
- 2-amino-6-(p-dimethylbenzamido)-4-isopropoxypteridine (example 40) : MS 353 ($[M+H]^+$, 75), 311 ($[M+H\text{-propene}]^+$, 100) ;
- 2-amino-6-(3-thienyl)-4-isopropoxypteridine (example 41) :.MS 288 ($[M+H]^+$, 55), 246 ($[M+H\text{-propene}]^+$, 100) ;
- 2-amino-6-(p-cyanophenyl)-4-isopropoxypteridine (example 42) : MS 307 ($[M+H]^+$, 40), 265 ($[M+H\text{-propene}]^+$, 100) ;
- 2-amino-6-(p-benzoic acid methyl ester)-4-isopropoxypteridine (exam-ple 43): MS 340 ($[M+H]^+$, 75), 298 ($[M+H\text{-propene}]^+$, 100) ;
- 2-amino-6-(p-acetylphenyl)-4-isopropoxypteridine (example 44) : MS 324 ($[M+H]^+$, 55), 282 ($[M+H\text{-propene}]^+$, 100) ; and
- 2-amino-6-(3,4-dimethoxyphenyl)-4-isopropoxypteridine (example 45) : MS 342 ($[M+H]^+$, 100), 300 ($[M+H\text{-propene}]^+$, 60) .

Example 46 - synthesis of 2,6-diamino-5-nitroso-4-hydroxypyrimidine

To a solution of 2,6-diamino-4-hydroxypyrimidine (12.9 g, 102.2 mmoles) in 200 ml of a 10% acetic acid solution in water at 80 °C was added dropwise a solution of $NaNO_2$ (7.05 g, 102.2 mmoles) in 20 ml water. A pink precipitate was formed, which was further stirred for 1 hour at 80 °C. The reaction mixture was cooled down in the refrigerator overnight. The precipitate was filtered off and dried over P_2O_5, providing the title compound as a pink powder (15.43 g, yield: 97%). The spectral data are in accordance with literature data (Landauer et al. in *J. Chem. Soc.* (1953) 3721-3722).

Example 47 - synthesis of 2,5,6-triamino-4-hydroxypyrimidine

A suspension of the compound of example 46 (15 g, 96.7 mmoles) in an ammonium sulfide solution (20 % in water, 200 ml) was stirred overnight at 50 °C. The reaction mixture was cooled down in the refrigerator and the precipitate was filtered off, providing the title compound as a yellow powder (11.33 g, yield: 83 %). The spectral data are identical with literature data (Landauer et al. cited *supra*).

Example 48 - synthesis of 2-amino-6-(3,4-dimethoxyphenyl)pterine

To a boiling solution of the compound of example 47 (2.4 g, 17 mmoles) in methanol (100 ml, with 0.9 N HCl) was added dropwise a solution of 3,4-dimethoxyphenylglyoxal mono-oxime (3.8 g, 18 mmoles) in methanol (100 ml). The reaction mixture was heated under reflux for 4 hours. The precipitate formed was filtered off, washed with water, then ethanol and diethyl ether, and dried over P_2O_5 under vacuum, providing the title compound as a yellow powder (4.33 g, yield: 85 %). This compound was further characterized by the following spectra:

- ^{1}H-NMR (500 MHz, TFA): δ 4.11 (3 H, s), 4.07 (3 H, s), 7.21 (1 H, d), 7.78 (1 H, dd), 7.81 (1 H, d) and 9.32 (1 H, s) ppm ;
- ^{13}C-NMR (125 MHz, TFA): δ 56.39, 56.7, 111.94, 113.21, 123.22, 127.41, 127.91, 145.92, 149.39, 150.46, 152.47, 153.15, 155.13 and 161.59 ppm .

Example 49 - synthesis of 2-acetylamino-6-(3,4-dimethoxyphenyl)pterine

A suspension of the compound of example 48 (10.46 g, 35 mmoles) in acetic anhydride (600 ml) and acetic acid (200 ml) was refluxed for 1 hour until a clear solution was formed. By cooling down the reaction mixture in the refrigerator, the precipitate formed was filtered off, washed with ethyl acetate and diethyl ether, and then dried over P_2O_5 under vacuum, providing the title compound as a yellow powder (9.19 g, yield: 77 %). This compound was further characterized by the following spectra :

- MS: *m/z* (%): 300 ([M+H]$^+$, 100) ;
- ^{1}H-NMR (200 MHz, DMSO-d_6): δ 2.22 (3 H, s), 3.84 (3 H, s), 3.87 (3 H, s), 7.14 (1 H, d), 7.75 (2 H, m) and 9.51 (1 H, s) ppm .

Example 50 - synthesis of 2-acetylamino-4-(1,2,4-triazolyl)-6-(3,4-dimethoxy-phenyl)pteridine

To a solution of phosphorus oxychloride (1.68 ml, 18 mmoles) and 1,2,4-triazole (4.96 g, 72 mmoles) in dry pyridine (110 ml) was added the compound of example 49 (2.45 g, 7.18 mmoles). The suspension was stirred at room temperature for 4 hours. The precipitate was filtered off, washed with pyridine, toluene and diethyl ether. The resulting solid was dried over P_2O_5 under vacuum, providing the title compound as a yellow powder (2 g, yield: 80 %) which afforded the following mass spectrum: 392 ([M+H]$^+$, 100) .

Examples 51 and 52 - synthesis of 2-amino-4-mercaptoethyl-6-(3,4-dimethoxyphenyl)pteridine and 2-amino-4-mercaptoisopropyl-6-(3,4-dimethoxyphenyl) pteridine

To a suspension of the compound of example 50 (0.25 mmole, 100 mg) in dioxane (5 ml) was added 1 mmole of either ethanethiol (example 51) or isopropanethiol (example 52) and sodium (12 mg, 0.5 mmole). The suspension was stirred for 24 hours at room temperature. The solvent was concentrated *in vacuo* and the residue purified by flash chromatography (silica, using a CH_3OH/CH_2Cl_2 mixture (5:95) as an eluent), followed by purification by preparative TLC, providing the pure title compounds as yellow powders with yields ranging from 20 to 30%. Both compounds were characterized by their mass spectrum as follows :

- 2-amino-4-mercaptoethyl-6-(3,4-dimethoxyphenyl) pteridine: 344 ([M+H]$^+$, 100) ;
- 2-amino-4-mercaptoisopropyl-6-(3,4-dimethoxyphenyl) pteridine: 357 ([M+H]$^+$, 100) .

Example 53 - synthesis of a mixture of 2,4-diamino-6-(p-methoxyphenyl) pteridine and 2,4-diamino-7-(p-methoxyphenyl)pteridine

2,4,5,6-tetra-aminopyrimidine (10 mmoles, 1.4 g) was dissolved in water (50 ml) and the pH was adjusted to 9 with ammonium hydroxide. A solution of 4-methoxyphenylglyoxal (11

mmoles, 1.8 g) in ethanol (10 ml) was added dropwise and the solution was refluxed for 1 hour. The yellow precipitate formed was filtered off and washed with water, ethanol and diethyl ether. NMR analysis reveals the obtention of a mixture (1.2 g, 45 % yield) consisting of 87 % of 2,4-diamino-7-(p-methoxyphenyl)pteridine and 13 % of 2,4-diamino-7-(p-methoxyphenyl)pteridine. ^{1}H-NMR (500 MHz, TFA): δ 4.04 (3 H, s), 4.08 (3 H, s), 7.15 (2 H, d), 7.25 (2 H, d), 8.19 (2 H, d), 8.30 (2 H, d), 9.27 (1 H, s) and 9.37 (1 H, s) ppm .

Example 54 - synthesis of a mixture of 2-amino-6-(p-methoxyphenyl)pterin and 2-amino-7-(p-methoxyphenyl)pterin

The mixture obtained in example 53 (1.2 g, 4.5 mmoles) was sus-pended in NaOH 1 N (80 ml) and refluxed till a solution was obtained . The hot solution was treated with acetic acid till pH 5, then cooled down and the resulting precipitate was filtered off and washed with water, ethanol and diethyl ether, providing a mixture of 2-amino-6-(p-methoxyphenyl)pterin and 2-amino-7-(p-methoxyphenyl) pterin as a yellow powder (1 g, yield: 82 %). Mass spectrum: 270 ($[M+H]^+$, 100) .

Example 55 - synthesis of 2-acetylamino-6-(p-methoxyphenyl)pterin and 2-acetylamino-7-(p-methoxyphenyl)pterin

A suspension of the mixture obtained in example 54 (7.43 mmoles, 2 g) was suspended in a mixture of acetic anhydride (50 ml) and acetic acid (50 ml). The suspension was refluxed for 4 hours till a clear solution was obtained. Some insoluble material was filtered off and the solution was partly evaporated till precipation starts. Further precipitation was achieved overnight in the refrigerator. The resulting precipitate was filtered off and washed with ethyl acetate and diethyl ether, providing a mixture of 2-acetylamino-6-(p-methoxyphenyl)pterin and 2-acetylamino-7-(p-methoxyphenyl)pterin as a yellow powder (2.1 g, 91 % yield). Mass spectrum: 312 ($[M+H]^+$, 100) .

Example 56 - synthesis of 2-acetylamino-4-(1,2,4-triazolyl)-6-(p-methoxy-phenyl) pteridine and 2-acetylamino-4-(1,2,4-triazolyl)-7-(p-methoxyphenyl) pteridine

To a suspension of the mixture obtained in example 55 (1.5 g, 4 mmoles) in dry pyridine (100 ml) was added 1,2,4-triazole (830 mg, 12 mmoles) and 4-chlorophenyl phosphorodichloridate (1 ml, 6 mmoles). The suspension was stirred for 2 days at room temperature under nitrogen. The solvents were removed *in vacuo*. The solid material was suspended in dichloromethane and washed with 2 % HCl. Evaporation of the solvents provided a mixture of 2-acetylamino-4-(1,2,4-triazolyl)-6-(p-methoxyphenyl) pteridine and 2-acetylamino-4-(1,2,4-triazolyl)-7-(p-methoxyphenyl)pteridine .

Example 57 - synthesis of 2-amino-4-isopropoxy-7-(p-methoxyphenyl) pteridine

To a suspension of the mixture obtained in example 56 (180 mg, 0.50 mmole) in isopropanol (8 ml) was added sodium (23 mg, 1 mmole). The suspension was stirred at room

temperature overnight. The solvents were evaporated and the residue was purified by preparative TLC (silica, using a methanol/CH_2Cl_2 (7:93) mixture as the eluent). At this stage, both regio-isomers obtained were separated, thus providing the pure title compound as a yellow powder (yield: 45 %) which was further characterized by its mass spectrum: 312 ($[M+H]^+$, 65), 270 ($[M+H\text{-propene}]^+$, 100) .

Example 58 - synthesis of 2-amino-4-isopropoxy-7-(3,4-dimethoxyphenyl) pteridine

The sequence of reactions described in examples 53 to 57 was followed, however starting from 3,4-dimethoxyphenylglyoxal instead of 4-methoxyphenylglyoxal in the first step. This provided 2-amino-4-isopropoxy-7-(3,4-dimethoxyphenyl) pteridine, a compound which was further characterized by its mass spectrum: 342 ($[M+H]^+$, 55), 300 ($[M+H\text{-propene}]^+$, 75) .

Example 59 - synthesis of 2-amino-4-ethoxy-7-(3,4-dimethoxyphenyl) pteridine

The sequence of reactions described in examples 53 to 57 was followed, however starting from 3,4-dimethoxyphenylglyoxal instead of 4-methoxyphenylglyoxal in the first step, and from ethanol instead if isopropanol in the last step. This provided 2-amino-4-ethoxy-7-(3,4-dimethoxyphenyl) pteridine, a compound which was further characterized as follows :

- MS: 328 ($[M+H]^+$,100), 300 ($[M+H\text{-ethene}]^+$, 40) ;
- ^{1}H-NMR (500 MHz, DMSO-d_6): δ 1.44 (3 H, t), 3.86 (3 H, s), 3.88 (3 H, s), 4.54 (2 H, q), 7.13 (1 H, d), 7.16 (2 H, br s), 7.85 (1 H, d), 7.88 (1 H, dd) and 9.06 (1 H, s) ppm ;
- ^{13}C-NMR (125 MHz, DMSO-d_6): δ 14.25, 55.67, 55.76, 63.08, 110.39, 111.89, 121.13, 121.25, 128.24, 136.87, 149.28, 151.62, 155.82, 156.72, 162.03 and 166.70 ppm .

Example 60 - synthesis of 2-amino-4-methoxy-7-(3,4-dimethoxyphenyl) pteridine

The sequence of reactions described in examples 53 to 57 was followed, however starting from 3,4-dimethoxyphenylglyoxal instead of 4-methoxyphenylglyoxal in the first step, and from methanol instead if isopropanol in the last step. This provided 2-amino-4-ethoxy-7-(3,4-dimethoxyphenyl) pteridine, a compound which was further characterized by its mass spectrum: 314 ($[M+H]^+$,100), 300 ($[M+H\text{-methane}]^+$, 20).

Example 61 – lymphocyte activation tests

Pteridine derivatives were first dissolved (10mM) in dimethylsulfoxide (hereinafter referred as DMSO) and further diluted in culture medium before use for the following *in vitro* experiments. The commercially available culture medium consisted of RPMI-1640 + 10% foetal calf serum (FCS). Some pteridine derivatives described the previous examples were tested in the following lymphocyte activation tests:

Mixed Lymphocyte Reaction

Peripheral blood mononuclear cells (hereinafter referred as PBMC) were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (Nycomed, Maorstua, Norway). Allogeneic PBMC or Eppstein-Barr Virus-transformed human

B cells [commercially available under the trade name RPMI1788 (ATCC name CCL156)] which strongly express B7-1 and B7-2 antigens were used as stimulator cells after irradiation with 30 Gy. MLR was performed in triplicate wells. After 5 days incubation at 37°C, 1 µCi [^{3}H]-thymidine was added to each cup. After a further 16 hours incubation, cells were harvested and counted in a ß-counter. Inhibition of proliferation by a compound (drug) described in some of the previous examples was counted using the formula:

$$\% \text{ inhibition} = \frac{(\text{cpm} + \text{drugs}) - (\text{cpm cult. med})}{(\text{cpm} - \text{drugs}) - (\text{OD cult. med})} \times 100$$

wherein cpm is the thymidine count per minute. The MLR assay is regarded by those skilled in the art as an *in vitro* analogue of the transplant rejection since it is based on the recognition of allogeneic major histocompatibility antigens on the stimulator leukocytes, by responding lymphocytes.

Assays for CD3 and CD 28

T cells were purified by removing non-T cells. Briefly, monocytes were removed by cold agglutination. The resulting lymphoid cells were further purified by a cell enrichment immunocolumn [Cellect Human T commercially available from Biotex, Edmonton, Alberta, Canada)] by a process of negative selection. More than 95% of the B cells were removed with this procedure. After depletion, the resulting T cell preparation was highly purified, i.e. these cells could not be activated by phytohaemagglutinin (PHA) or rIL-2 alone at concentrations capable of stimulating RBMC prior to deletion.

Highly purified T cells (10^6/ml) were stimulated by immobilized anti-CD3 or anti-CD28 monoclonal antibodies (hereinafter referred as mAb) in the presence of phorbol myristate acetate (hereinafter referred as PMA). Anti-CD3 mAb (available from CLB, Amsterdam, Netherlands) were fixed on a 96-microwell plates by incubating the wells with 50 µl of mAb solution (1/800 dilution in culture medium). For anti-CD28 mAb (available from CLB, Amsterdam, Netherlands) 50 µl (1/650 dilution in culture medium) was added directly to the wells. Further, a 20 µl PMA (commercially available from Sigma, St. Louis, Missouri, USA) solution (final concentration: 0.5 ng/ml) was added. Subsequently, 20 µl of a pteridine derivative described in the previous examples were added by serial dilution in triplicate wells. Finally 100 µl of the T-cell suspension (10^6/ml) was added. After 48-hour incubation at 37°C in 5% CO_2, 20 µl of a bromo-deuridine (hereinafter referred as BrdU) 100 µM solution (commercially available as Cell Proliferation Elisa from Boehringer-Mannheim Belgium) was added to each well. After a further overnight incubation, T-cell proliferation was measured using a colorimetric immuno-assay for qualification of cell proliferation based on the incorporation of BrdU during DNA synthesis. Optical density (hereinafter referred as OD) was measured by a Behring EL311 plate reader at 450 nm (reference wavelength: 690 nm). Inhibition of proliferation by the pteridine derivative (drug) was counted while using the formula:

$$\% \text{ inhibition} = \frac{(OD + drugs) - (OD \text{ cult. med})}{(OD - drugs) - (OD \text{ cult. med})} \times 100$$

Table 1 (wherein ND means not determined) below shows the IC_{50} values for various pteridine derivatives in the MLR test and in the CD3 and CD28 assays. The IC_{50} value represents the lowest concentration of the pteridine derivative (expressed in µmole/l) that resulted in a 50% suppression of the MLR or a 50% reduction in T-cell proliferation (for the CD3 and CD28 assay).

TABLE 1

Example n°	MLR	CD 3	CD28
15	8.3	20	7.0
16	> 10	9.0	4.0
18	10	10	0.8
20	ND	15	0.7
25	8.2	10	10
30	0.9	0.7	0.7
32	10	ND	ND
45	0.4	6.7	1.7

ND: not determined

The above data show that, whereas known immunosuppressant drugs like CyA are known to be active only in the CD3 assay, the pteridine derivatives according to the present invention were also active in the CD28 assay which is Ca^{2+}-calmodulin resistant. The CD28 pathway is a so-called cosignal pathway which is important for inducing energy and even tolerance in T-cells.

Example 62 - TNF-α and IL-1 β assays

Peripheral blood mononuclear cells (herein referred as PBMC), in response to stimulation by lipopolysaccharide (LPS), a gram-negative bacterial endotoxin, produce various chemokines, in particular human TNF-α and IL-1 β. Inhibition of the activation of PBMC can therefore be measured by the level of suppression of the production of TNF-α or IL-1 β by PBMC in response to stimulation by LPS.

Such inhibition measurement was performed as follows: PBMC were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (commercially available from Nycomed, Norway). LPS was then added to the PMBC suspension in complete medium (10^6 cells /ml) at a final concentration of 1 µg/ml. The pteridine derivative to be tested was added at different concentrations (0.1 µM, 1 µM and 10 µM) and the cells were incubated at 37°C for 72 hours in 5% CO_2. The supernatants were collected, then TNF-α and/or IL-1 β concentrations were measured with respectively an anti-TNF-α antibody or an anti-IL-1 β antibody in a sandwich ELISA (Duo Set ELISA human TNFα, commercially available from R&D Systems, United Kingdom). The colorimetric reading of the ELISA was measured by a Multiskan RC plate reader (commercially available from ThermoLabsystems, Finland) at 450 nm (reference wavelength: 690 nm). Data analysis was performed with Ascent software 2.6. (also from ThermoLabsystems, Finland): a standard curve (recombinant human TNFα) was drawn and the amount (pg/ml) of each sample on the standard curve was determined.

The % suppression of human TNFα production or human IL-1 β by the pteridine derivatives of the invention (drugs) was calculated using the formula:

$$\% \text{ suppression} = \frac{\text{pg/ml in drugs} - \text{pg/ml in cult. med.}}{(\text{pg/ml in cult. med.} + \text{LPS}) - \text{pg/ml cult. med.}}$$

Table 2 below shows the IC_{50} values (expressed in µM) of the tested pteridine derivatives in the TNF-α assay.

TABLE 2

Example n°	TNF-α	Example n°	TNF-α
6	8.0	30	6.6
10	4.0	32	6.7
12	9.1	34	7.5
13	4.5	36	9.1
14	10.0	37	6.6
15	10.0	38	6.2
19	8.5	39	10.0
24	8.1	41	7.6
25	8.5	42	6.3
27	6.8	44	9.1
28	10.0	45	3.5

Example 63 – inhibition of the release of cytokines involved in asthma

2-amino-4-morpholino-6-(3,4-dimethoxyphenyl)pteridine (a compound known from WO 00/39129) was tested, according to experimental procedures well established in the art, in the inhibition of two cytokines, IL-5 and IL-10, known to be involved in the development of asthma and other forms of severe allergic conditions. At the concentration of 0.1 µM/l, i.e. the IC_{50} value of this derivative in the MLR test, the said derivative inhibited the release of IL-5 and IL-10 by 70% and 39%, respectively.

Example 64 – inhibition of the metastasis of melanoma cells in mice

C57BL/6 mice were injected with $1.5x10^6$ B16BL/6 melanoma cells and were divided into 2 groups. Five days after the injection of the tumor cells, the two groups of animals were administered intraperitoneously, three times a week for two subsequent weeks, either (treated group) 20 mg/kg of 2-amino-4-morpholino-6-(3,4-dimethoxyphenyl)pteridine (a compound known from WO 00/39129) or (control group) the vehicle (5% DMSO in phosphate buffered saline). At the end of the experiment, all surviving tumor-bearing mice were sacrificed and examined in order to assess the absence or presence of the black metastases in inguinal and/or para-aortic lymph nodes (macroscopic metastasis). The findings were confirmed by histology, and the experiment done twice. The pooled results of the two experiments show that 80% of animals from the control group had metastases whereas only 33% of animals from the treated group had metastases.

Example 65 - *in vivo* leukocyte activation and immunosuppression whole blood assay

In response to non-self antigens (e.g. microbial antigens, allo-antigens, altered self antigens and auto-antigens), immune cells such as lymphocytes, NK cells, monocytes/macrophages and dentritic cells are activated to generate antigen-reactive effector cells. Activated immune cells up-regulate a number of pro-inflammatory genes encoding cytokines (e.g. IL-2, IFN-γ, and TNF-α), chemokines (e.g. monocyte chemo-attractant protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1) and RANTES), and cell surface molecules. These leukocyte surface molecules may include activation associated molecules (e.g. CD69, the lymphocyte early activation molecule, and CD71, the transferin receptor), adhesion molecules (e.g. ICAM-1, LFA-1 and L-selectins), co-stimulatory molecules (e.g. CD28, B7-1 and CD40L) and receptors for cytokines and chemokines (e.g. IL-2R and CCR1).

Immunomodulatory therapies are active by interfering with the responses of the immune system. Reliable, easy, fast and quantitative methods for monitoring the evolution of the leucocyte surface and cytoplasma phenotypes during activation are critical in evaluating the functional competence of the immune cells (e.g., during HIV infection), as well as the efficacy of immunoregulatory therapies in order to achieve optimal therapeutic effects and minimal side effects. Moreover, examination of drug action on leukocyte activation is able to provide critical evaluation of pharmacokinetic and pharmacodynamic effects of immunomodulatory agents. Within this context, the following provides a useful *in vivo* leukocyte activation and immunosuppression whole blood assay (hereinafter referred as WBA).

Inbred mice (e.g. AKR strain), weighting from 20 to 30g, were stimulated by intra-peritoneal injection with mitogens specific for T cells (e.g. Concanavalin A, hereinafter referred as ConA, 500 μg per mouse), B cells or monocytes/macrophages (e.g. LPS 20 μg per mouse). The pteridine derivatives of example 30 and, respectively, example 45, were administered intraperito-neously (30 mg/kg per day, administered 2 hours before ConA injection). Peripheral blood was collected 24, 36, 48, and respectively 72 hours after stimulation for immune fluorescence staining and FACScan analysis.

FACScan analysis proceeded as follows: heparinized whole blood (50 μl) was removed of red blood cells by incubation with a lysing buffer. The remaining leukocytes were double-stained with phycoerythrin-conjugated antibodies specific for T cells (e.g. T cell receptor TCR), B cells (e.g. CD45R/B220 (commercially available from Pharmingen) or monocytes/macrophages (e.g. F4/80) (commer-cially available from Serotec) in combination with FITC-conjugated antibodies against various cell surface markers such as CD69 (early activation molecule), CD25 (IL-2 receptor), CD71 (transferring receptor), B7-1/2 (ligand for the co-stimulatory molecule CD28) and CD11b (complement receptor 3). Cells were examined by flow cytometry using CellQuest software (commercially available from Decton Dickinson).

Peripheral blood T cells expressed an up-regulated level of CD69, representing up to 3% of TCR^+,$CD69^+$ cells respectively 1 and 2 days following ConA injection, as compared to baseline

levels (0.5%). T cells from mice treated with the pteridine derivatives of examples 30 and 45 show a remarkable reduction of this response to 1.6% and 2.4% respectively.

Peripheral blood T cells showed a rapid up-regulation of CD71 from baseline level (4.8%) to 9.3% and 5.5% after 1 day and 2 days, respectively. The pteridine derivatives of examples 30 and 45 suppressed ConA-stimulated CD71 up-regulation on T cells to baseline levels.

Peripheral blood T cells expressed increased levels of IL-2R with up to 3% of TCR+, IL-2R+ cells 1 and 2 days following ConA stimulation, with peak levels after day 2. Administration of the pteridine derivatives of examples 30 and 45 effectively suppresses this effect by reducing expression to 1.5% and 1.7%, respectively.

Up to 6.6 % of peripheral blood T cells displayed an up-regulation of the CD134 molecule expression within 2 days following ConA stimulation, i.e. a two-fold increase of baseline levels (2.2%). Treatment with the pteridine derivatives of examples 30 and 45 suppressed this response to 3.5% and 4.2%, respectively.

This FACScan-based cell surface phenotype analysis for *in vivo* leukocyte activation, namely for an *in vivo* whole blood assay, demonstrates the immunosuppressive effects of the pteridine derivatives of examples 30 and 45 *in vivo*. This observation is consistent with the above experimental results *in vitro* showing that both compounds inhibit T cell activation and proliferation in mixed lymphocyte culture, and in the CD3 and CD28 assays.

In vivo WBA by injecting immune activators, followed by FACS analysis of leukocyte surface phenotypes in the whole blood can be applied to monitor *in vivo* activation of different types of leukocytes such as T cells, B cells, monocytes/macrophages, and dentritic cells. This technology allows simple, rapid, direct, stable and quantitative evaluation of the immune competence of the said leukocytes, as well as pharmacokinetic and pharmacodynamic profiles of the pteridine derivatives used as immunomodulatory agents *in vivo*.

Despite various *in vitro* models are used for the discovery of new immunosuppressive drugs, there are essentially no ideal animal models that are simple, fast, and cost-effective. Models of organ transplantation (e.g. hearts, kidney and skin) in rodents are limited by technical difficulty, and cannot be used for primary screening of a large numbers of candidate drugs. Although recently a mouse model of drug-mediated immunosuppression based on rejection of an allogeneic subcutaneous tumor was reported, several significant limitations should be considered. For example first, the host immune responses against this specific tumor seems to be T cell-dependent, and thus the activity of a drug on other cell types such as B cells, monocytes/macrophages and dentritic cells cannot be evaluated by using this model. Secondly, immunosuppressive drugs with anti-proliferation activity (e.g. rapamycin) may play a direct role on tumor angiogenesis and tumor cell growth. Thirdly, the feature of chronic rejection of the tumor

(e.g. after 14 days) prevents from evaluating the pharmacodynamic properties of drugs. Finally, this animal tumor rejection model is inaccurate (e.g. quantified by size measurement) and time-costly (at least 14 days).

FACScan-based *in vitro* WBA has been used to investigate the pharmaco-dynamic effects of immunomodulatory drugs administrated *in vivo*, but only by taking blood from treated recipients and stimulating their lymphocytes *in vitro*. These methods show remarkable advantages as compared to the purified peripheral blood mononuclear cell (PBMC) cultures in terms of maintaining cell viability, as well as the effects within the whole blood of immunomodulatory drugs previously administrated *in vivo*. However, in vitro WBA has important flaws. First, the evaluation of the drug effects uses an *in vitro* activation system, including manipulation, dilution and stimulation of the blood, and subsequent culture of the blood in the presence of an exogenous serum (e.g. foetal calf serum). This *in vitro* activation system may not necessarily and accurately reflect the effects of drugs on leukocyte activation *in vivo*, nor does this assay give information on the absorption or metabolite production of drugs that may occur *in vivo*. Secondly, *in vitro* stimulation of the whole blood by mitogens (e.g. Con A, PHA or LPS) causes aggregation of activated leukocytes, platelets and red blood cells, as well as generation of spontaneous fluorescence by *in vitro* damaged cells. These alterations frequently interfere with the FACS analysis. Thirdly, plastic adhesion of monocytes/macrophages during *in vitro* culture may induce additional steps in order to examine these cell types. Finally, *in vitro* culture of leucocytes is expensive, time consuming, and poorly reproducible. The above described *in vivo* WBA assay is able to overcome the above mentioned problems and may be characterized as follows:

- leukocyte stimulation is performed *in vivo* (e.g. by *in vivo* injection of leucocyte stimulating agents such as, but not limited to, ConA or LPS, thus enabling the full *in vivo* evaluation of the effect of drugs on leucocyte activation in physiological conditions,
- various stimuli can be used that are specific for different cell types, including T cells (e.g. ConA, anti-CD3 antibodies and alloantigens), B cells (e.g. T cell-independent xeno-antigens, sheep red blood cells, TNP-ficoll, TNP-BA, anti-IgM antibodies and LPS) and monocytes/macrophages (e.g. LPS, TNF-α and IFN-γ). Given the time-dependent nature of the expression of cell surface antigens, the effects of drugs can be studied on early as well as on late leucocyte activation, e.g. by analyzing the expression of early (e.g. CD69 and CD71) or late (e.g. IL-2R and CD134) appearing antigens,
- by varying the time of administration of a drug prior to or after *in vivo* injection of the leucocyte stimuli, the pharmacodynamic profile of the said drug can be determined. Similarly, the effects and the duration of activity of a drug via different routes of administration, e.g. intraperitoneously, intramuscularly and orally, can be studied,
- by using the same test in immunodeficient animals (e.g. T cell-deficient nude mice), the effect of a drug on selected immune phenomena can be investigated (e.g. on in vivo T

cell-independent B cell activation in nude mice; T cell activation in T cell-reconstituted SCID mice that have congenital defect on both T and B cells),

- peripheral leukocytes from different sources, such as spleen, lymph nodes and peritoneal cavity, can be tested,
- in combining FACScan-based intracellular phenotype analysis with e.g. intracellular cytokine staining, or DNA synthesis by BrdU or 5- and 6-carboxyfluorescein diacetate succinimidyl ester (CFSE) incorporation, or cell cycle studies, the information obtained can be further broadened, and
- the method is able to help in elucidating the mechanism underlying *in vivo* up-regulation of expression of certain activation molecules, such as CD69, CD25, CD71, CD134 and B7-1/2, in various types of peripheral blood leucocytes.

Example 66 - synthesis of 4,5,6-triamino-2-methylmercaptopyrimidine dihydro-chloride

To a suspension of a 2-methylmercapto-4,5,6-triamino-pyrimidine sulfate (44.3 mmole), which may be prepared and characterised for instance as disclosed by Taylor et al. in J. Am. Chem. Soc. (1952) 74:1644-1647, in water (135 ml) at 80 °C was added dropwise a solution of barium chloride dihydrate (39.8 mmole) in water (25 ml). The suspension was stirred for 30 minutes at 80 °C. The reaction mixture was cooled down and barium sulfate was filtered off over Celite. The filtrate was evaporated in vacuo and co-evaporated with toluene yielding the title compound as a yellow powder (10.2 g, 94 % yield).

Example 67 - synthesis of 4-amino-2-methylmercapto-6-(3,4-dimethoxy-phenyl) pteridine

To a suspension of 4,5,6-triamino-2-methylmercaptopyrimidine dihydrochloride (7.42 mmole, 1.81 g) in methanol (20 ml) was added a solution of 3,4-dimethoxyphenylglyoxaloxime (5.94 mmole, 1.24 g) in methanol. The resulting reaction mixture was refluxed for 3 hours. The reaction mixture was neutralised with concentrated aqueous ammonia until pH 9 was reached. The resulting precipitate was filtered off and further purified by flash chromatography (silica, using an ethyl acetate / hexane mixture in a 4:6 ratio) yielding the pure title compound as a yellow powder which was characterised as follows: MS: m/z (%) 330 ($[M+H]^+$, 100), 681 ($[2M+Na]^+$, 30); UV (MeOH, nm): 292, 397.

Example 68 - synthesis of 2,4-di-(thienyl-2-methylamino)-6-(3,4-dimethoxy-phenyl) pteridine

A solution of the compound of example 67 (100 mg, 0.304 mmole) in 2-thiophenemethylamine (12 ml) was refluxed overnight. The solvents were removed in vacuo and the residue was purified first by flash chromatography (silica, gradient from 2:98 to 3:97 CH_3OH /CH_2Cl_2) and then by preparative TLC (silica, EtOAc/hexane 1 :1) yielding the title

compound as a yellow powder (85 mg, 57 %) which was characterised as follows : MS: m/z (%): 491 ($[M+H]^+$, 100); UV (MeOH, nm): 229, 296, 414 .

Example 69 - synthesis of 2,5,6-triamino-4-morpholino-pyrimidine dihydro-chloride

In a 10 L 4-neck flask equipped with a mechanical stirrer, a thermometer and a heating mantle was placed 2,6-diamino-4-chloropyrimidine (870.5 g; 6.0 mol) in water (3190 ml). To the stirred resulting white suspension was added morpholine (1113 g; 12.8 moles) in one portion. The temperature raised from 14 to 28°C and the mixture was heated until at 75°C a clear solution was obtained and refluxed for 16 hours. After complete conversion of the starting material (controlled by HPLC analysis) the reaction mixture was cooled to room temperature and $NaNO_2$ (465 g, 6.74 mole) was added in three portions. No heat effect was observed but the product precipitated out of the mixture. During subsequent dropwise addition of acetic acid (600 g, 9.0 moles) the mixture became so thick that faster stirring and additional water (850 mL) were necessary to achieve a well mixable suspension. A temperature raise from 15 to 28°C was measured. After stirring for 2hours, HPLC analysis showed complete conversion. The mixture was then allowed to cool down to room temperature and transferred to a 20L flask. To the acid (pH 4) purple slurry was added water (3500 mL) and then an aqueous NaOH solution (165 g in 825 mL) in order to neutralise to pH 7. The suspension was cooled down to 10-12°C in an ice-water bath and $Na_2S_2O_4$ (4.68 kg, 26.9 moles) was added in portions of about 500 g at 5 minutes intervals. A temperature raise to 30°C was observed. The mixture was left stirring overnight at room temperature before being analysed (HPLC: 99.3% conversion). The suspension was filtered over filter cloth and the wet cake was washed with 2.77 L water leaving 1.5 kg of wet material which was slurried in isopropanol (10.2 L) and to the suspension was added dropwise 36% HCl/H_2O (1.16L) during which the temperature raised from 15 to 30°C. Subsequent heating of the slurry to 40°C afforded an almost clear solution and crystallisation started suddenly showing an exotherm to 50°C. The suspension was cooled down to room temperature before the suspension was filtered over filter cloth. The wet cake was again slurried in isopropanol (5.0 L), again filtered over filter cloth and dried at 40°C in vacuo (200 mbar) for several days. According to this procedure was obtained 1.2 kg (yield 54.1%) of 2,5,6-triamino-4-morpholino-pyrimidine dihydrochloride with a purity above 99.9% and characterised as follows: UV (MeOH, nm): 225, 254.

Example 70 - synthesis of 4-acetamidophenylglyoxalmonoxime

SeO_2 (34.18 g, 0.308 mole) and 4-acetamidoacetophenone (49.62 g, 0.28 mole) were suspended in a mixture of dioxane (250 ml) and water (10 ml) and the solution was heated to 100°C for 16 hours. The hot solution was filtered on a paper filter and the filtrate was evaporated to dryness. The oily residue was then partitioned between $CHCl_3$ (400 ml) and a saturated solution of sodium hydrogen-carbonate (200 ml). A precipitation occurs in the aqueous layer. The

organic phase was collected and the aqueous layer was filtered over a fritted glass. The solid (4-acetamidoglyoxaldehyde) was washed with water and kept for the next step. The aqueous layer was extracted several times with $CHCl_3$ and all fractions were collected and evaporated to dryness. The oily residue and the precipitate were put together and suspended in a mixture of water (240 ml) and MeOH (60 ml). Then acetonoxime (20.46 g, 0.28 mole) was added and the pH was adjusted to a range of 3 - 4 with 1 N HCl. The solution was heated to 70 °C for 1 hour, then cooled to 0 °C and the resulting crystals collected. After washing with cold water, then diethylether, drying in a vacuum dessicator over P_2O_5 afforded 33.6 g of the desired compound (yield 58%) as yellowish crystals with a purity of 95%, which were characterised as follows: MS: m/z (%): 207 ($[M+H]^+$, 100); UV (MeOH, nm): 241.6, 278.3.

Example 71 - synthesis of 3-acetamidophenylglyoxalmonoxime

SeO_2 (36.62 g, 0.33 mole) and 3-acetamidoacetophenone (53.16 g, 0.30 mole) were suspended in a mixture of dioxane (250 ml) and water (10 ml) and the solution was heated to 100°C for 16 hours. The hot solution was filtered on a paper filter and the filtrate was evaporated to dryness. The oily residue was then suspended in a mixture of water (240 ml) and MeOH (60 ml) and acetonoxime (21.93 g, 0.30 mol) was added and the pH was adjusted to a range of 3 - 4 with 6 N HCl. The solution was heated to 70 °C for 2 hours and after cooling, the brownish precipitate was filtered and re-crystallised from toluene to afford 45.0 g of the desired compound (yield 72%) with a purity of 98%, which was characterised as follows: MS: m/z (%): 207 ($[M+H]^+$, 100), 229 ($[M+Na]^+$,80); UV (MeOH, nm): 239.2.

Example 72 - synthesis of 2-amino-4-morpholino-6-(4-acetanilide) pteridine

2,4,5-triamino-6-morpholinopyrimidine dihydrochloride (14.1 g, 50 mmole) was refluxed in MeOH (300 ml) and the compound of example 70 (11.33 g, 55 mmole) in MeOH (150 ml) was added. The mixture was refluxed for 3 hours and after cooling, the yellowish precipitate was filtered, washed with water, methanol, ether and dried at 110°C for 3 hours to afford 15.7 g of the desired product (yield 86%) which, after re-crystallisation from DMF/H_2O, was obtained with a purity of 97% and characterised as follows: MS: m/z (%): 366 ($[M+H]^+$, 100), 753 ($[2M+Na]^+$,15); UV (MeOH, nm): 213, 307, 408.

Example 73 – synthesis of 2-amino-4-morpholino-6-(3-acetanilide) pteridine

Repeating the procedure of example 72 but starting from the compound of example 71, the title pteridine derivative (16.1 g) was obtained with a yield of 88% and a purity of 97%, and characterised as follows: MS: m/z (%): 366 ($[M+H]^+$, 100), 753 ($[2M+Na]^+$,80); UV (MeOH, nm): 220, 294, 402.

Example 74 - synthesis of 2-amino-4-morpholino-6-(4-aminophenyl) pteridine

The crude compound of example 72 (20 mmole) was refluxed in a mixture of MeOH/HCl 6N – 1/1 (400 ml) for 2 hours and cooled in an ice bath. The remaining solid was filtered and the pH was adjusted to 10 with NaOH (10N). The orange precipitate was filtered, washed with water, ethanol, ether and dried in a vacuum dessicator over P_2O_5 to afford 4.7 g (yield: 73%) of the desired compound as an orange solid with a purity of 98%, which was characterised as follows: MS: m/z (%): 324 ($[M+H]^+$, 100); UV (MeOH, nm): 216, 316, 422; ^1H NMR (200 MHz, in DMSO-d6, ppm): 3.78 (br s, 4H); 4.33 (br s, 4H); 5.54 (br s, 2H); 6.63 (br s, 2H); 6.67 (d, 2H, J = 8.4 Hz); 7.75 (d, 2H, J = 8.4 Hz); 9.15 (s, 1H).

Example 75 - synthesis of 2-amino-4-morpholino-6-(3-aminophenyl) pteridine

Repeating the procedure of example 74 but starting from the compound of example 73, the title pteridine derivative (4.88 g) was obtained with a yield of 74% and a purity of 99.34%, and characterised as follows: MS: m/z (%): 324 ($[M+H]^+$, 100); UV (MeOH, nm): 218, 292, 403.

Examples 76 to 82 - coupling reaction between a 2-amino-4-morpholino-6-(4-amino-phenyl) pteridine and a carboxylic acid chloride, sulfonyl chloride or carbamoyl chloride

The compound of example 74 (162 mg, 0.5 mmole) was suspended in dioxane (10 ml) and triethylamine was added (84 µl, 0.6 mmole). Then a suitable carboxylic acid chloride or sulfonyl chloride or carbamoyl chloride (0.55 mmole) was added and the mixture was stirred at room temperature until completion of the reaction. After evaporation to dryness, the crude resulting product was purified by flash chromatography on silica gel using a gradient of MeOH (1-5%) in dichloromethane. Yields varied from 50 to 80%. Using this procedure, the following pteridine derivatives were prepared:

- 2-amino-4-morpholino-6-(4-benzoylaminophenyl) pteridine (example 76), purity 98.94%, characterised as follows: MS: m/z (%): 428 ($[M+H]^+$, 100), 877 ($[2M+Na]^+$, 40); UV (MeOH, nm): 313, 408;
- 2-amino-4-morpholino-6-(4-phenoxyacetylaminophenyl) pteridine (example 77), purity 97.18%, characterised as follows: MS: m/z (%): 458 ($[M+H]^+$, 100); UV (MeOH, nm): 305, 407;
- 2-amino-4-morpholino-6-(4-propionylaminophenyl) pteridine (example 78), purity 98.86%, characterised as follows: MS: m/z (%): 380 ($[M+H]^+$, 100), 781 ($[2M+Na]^+$, 20); UV (MeOH, nm): 213, 307, 408;
- 2-amino-4-morpholino-6-(4-furoylaminophenyl) pteridine (example 79), purity 93.96%, characterised as follows: MS: m/z (%): 418 ($[M+H]^+$, 100); UV (MeOH, nm): 213, 316, 408;
- 2-amino-4-morpholino-6-(4-cyclohexanoylaminophenyl) pteridine (example 80), purity

96.91%, characterised as follows: MS: m/z (%): 434 ($[M+H]^+$, 100), 889 ($[2M+Na]^+$, 10); UV (MeOH, nm): 308, 408;

- 2-amino-4-morpholino-6-[4-(4-chlorobenzoyl)aminophenyl] pteridine (example 81), purity 96.48%, characterised as follows: MS: m/z (%): 462 ($[M+H]^+$, 100); UV (MeOH, nm): 313.9, 407; and
- 2-amino-4-morpholino-6-(4-benzyloxyacetylaminophenyl) pteridine (example 82), purity 98.45%, characterised as follows: MS: m/z (%): 472 ($[M+H]^+$, 100), 942 ($[2M+H]^+$, 5), 965 ($[2M+Na]^+$, 10); UV (MeOH, nm): 307, 407.

Examples 83 to 97 - coupling reaction between a 2-amino-4-morpholino-6-aminophenyl pteridine and a carboxylic acid chloride, sulfonyl chloride or carbamoyl chloride

The compound of example 74 or example 75 (162 mg, 0.5 mmole) was suspended in pyridine (10 ml). Then a suitable carboxylic acid chloride or sulfonyl chloride or carbamoyl chloride (0.55 mmole) was added and the mixture was stirred at room temperature until completion of the reaction. After evaporation to dryness, the crude resulting product was purified by flash chromatography on silica gel using a gradient of MeOH (1-5%) in dichloromethane. Yields varied from 20 to 80%. Using this procedure, the following pteridine derivatives were prepared:

- 2-amino-4-morpholino-6-(4-isonicotinoylaminophenyl) pteridine (example 83) was characterised as follows: MS: m/z (%): 429 ($[M+H]^+$, 100), 879 ($[2M+Na]^+$, 5); UV (MeOH, nm): 213, 313, 407; purity 95.01%;
- 2-amino-4-morpholino-6-(4-naphtoylaminophenyl) pteridine (example 84) was characterised as follows: MS: m/z (%): 478 ($[M+H]^+$, 100), 977 ($[2M+Na]^+$, 5); UV (MeOH, nm): 213, 313, 407; purity 95.01%;
- 2-amino-4-morpholino-6-(4-methylsulfonylaminophenyl) pteridine (example 85) was characterised as follows: MS: m/z (%): 502 ($[M+H]^+$, 100); UV (MeOH, nm): 301, 422; purity 96.81%;
- 2-amino-4-morpholino-6-(4-ethylsuccinylaminophenyl) pteridine (example 86) was characterised as follows: MS: m/z (%): 452 ($[M+H]^+$, 100), 925 ($[2M+Na]^+$, 15); UV (MeOH, nm): 213, 308, 408; purity: 98.98%;
- 2-amino-4-morpholino-6-[4-(4-methylbenzoate)aminophenyl) pteridine (example 87) was characterised as follows: MS: m/z (%): 486 ($[M+H]^+$, 100); UV (MeOH, nm): 236, 315, 407; purity 99.57%;
- 2-amino-4-morpholino-6-(3-benzoylaminophenyl) pteridine (example 88) was characterised as follows: MS: m/z (%): 428 ($[M+H]^+$, 100); UV (MeOH, nm): 216, 293, 402; purity: 96.46%;
- 2-amino-4-morpholino-6-(3-benzensulfonylaminophenyl) pteridine (example 89) was characterised as follows: MS: m/z (%): 464 ($[M+H]^+$, 100); UV (MeOH, nm): 216, 295, 402;

purity: 97.49%;

- 2-amino-4-morpholino-6-(3-phenoxyacetylaminophenyl) pteridine (example 90) was characterised as follows: MS: m/z (%): 458 ($[M+H]^+$, 100); UV (MeOH, nm): 219, 294, 402; purity 98.96%;
- 2-amino-4-morpholino-6-(3-isonicotinoylaminophenyl) pteridine (example 91) was characterised as follows: MS: m/z (%): 429 ($[M+H]^+$, 100); UV (MeOH, nm): 216, 294, 402; purity 93.92%;
- 2-amino-4-morpholino-6-(3-cyclohexanoylaminophenyl) pteridine (example 92) was characterised as follows: MS: m/z (%): 434 ($[M+H]^+$, 100); UV (MeOH, nm): 222, 245, 294, 402; purity 99.50%;
- 2-amino-4-morpholino-6-[3-(4-methylbenzoate)aminophenyl] pteridine (example 93) was characterised as follows: MS: m/z (%): 486 ($[M+H]^+$, 100); UV (MeOH, nm): 218, 238, 294, 402; purity 97.44%;
- 2-amino-4-morpholino-6-(3-ethylsuccinylaminophenyl) pteridine (example 94) was characterised as follows: MS: m/z (%): 452 ($[M+H]^+$, 100); UV (MeOH, nm): 222, 245, 294, 402; purity 94.16%;
- 2-amino-4-morpholino-6-(3-ethylmalonylaminophenyl) pteridine (example 95) was characterised as follows: MS: m/z (%): 438 ($[M+H]^+$, 100); UV (MeOH, nm): 220, 244, 294, 402; purity 90.89%;
- 2-amino-4-morpholino-6-(3-benzyloxyacetylaminophenyl) pteridine (example 96) was characterised as follows: MS: m/z (%): 472 ($[M+H]^+$, 100); UV (MeOH, nm): 216, 243, 294, 402; purity 99.70%; and
- 2-amino-4-morpholino-6-(3-ethylsulfonylaminophenyl)pteridine (example 97) was characterised as follows: MS: m/z (%): 4716 ($[M+H]^+$, 100); UV (MeOH, nm): 218, 295, 402; purity 92.54%.

Examples 98 to 101 - coupling reaction between 2-amino-4-morpholino-6-(3-aminophenyl) pteridine and various carboxylic acids

The compound of example 75 (323 mg, 1 mmole), a carboxylic acid (1.1 mmole) and DIEA (2 mmole) were suspended in dry DMF (10 ml) and benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate) was added (1.1 mmole). The mixture was stirred at room temperature until completion of the reaction and then diluted with water. The aqueous layer was extracted with chloroform and the organic layer was evaporated to dryness. The crude resulting product was purified on silica gel using a gradient of MeOH (1-5%) in dichloromethane. Yields varied from 20 to 50%. The following compounds were made according to this procedure:

- 2-amino-4-morpholino-6-[3-Boc-(L)-phenylalanine-aminophenyl] pteridine (example 98) was

characterised as follows: MS: m/z (%): 1141 ($[2M+H]^+$, 10), 571 ($[M+H]^+$, 100), 515 ($[M-tBu+H]^+$, 50), 471 ($[M-Boc+H]^+$, 10); UV (MeOH, nm): 213, 245, 294, 402; purity 96.72%;

- 2-amino-4-morpholino-6-[3-Boc-(D)-phenylalanine-aminophenyl] pteridine (example 99) was characterised as follows: MS: m/z (%): 571 ($[M+H]^+$, 100), 515 ($[M-tBu+H]^+$, 50), 471 ($[M-Boc+H]^+$, 10); UV (MeOH, nm): 213, 245, 294, 402; purity: 95.30%;
- 2-amino-4-morpholino-6-[3-Boc-(L)-tryptophane-aminophenyl] pteridine (example 100) was characterised as follows: MS: m/z (%): 610 ($[M+H]^+$, 100), 554 ($[M-tBu+H]^+$, 50), 510 ($[M-Boc+H]^+$, 10); UV (MeOH, nm): 220, 291, 402; purity 91.74%; and
- 2-amino-4-morpholino-6-[3-Boc-(D)-tryptophane-aminophenyl] pteridine (example 101) was characterised as follows: MS: m/z (%): 610 ($[M+H]^+$, 100), 554 ($[M-tBu+H]^+$, 50), 510 ($[M-Boc+H]^+$, 10); UV (MeOH, nm): 220, 291, 402; purity 84.25%.

Example 102 - synthesis of 2-amino-4-morpholino-6-(4-hydroxyphenyl) pteridine

To a solution of 2,5,6-triamino-4-morpholino-pyrimidine dihydrochloride salt (5.05 g, 17.8 mmole) in methanol (120 ml) was added 4-hydroxyphenylglyoxalmonoxime (2.95 g, 17.8 mmole). The reaction mixture was refluxed for 3 hours and then cooled down to room temperature. The yellow precipitate was filtered off, washed with water yielding the title compound as a chromatographically pure yellow powder which was further characterised as follows: MS: m/z (%): 671 ($[2M+Na]^+$, 5), 325 ($[M+H]^+$, 100); UV (MeOH, nm): 213, 302, 411.

Examples 103 to 111 - synthesis of 2-amino-4-morpholino-6-(4-alkoxy-phenyl) pteridines

To a solution of the compound of example 102 (0.65 mmole, 210 mg) in DMF was added K_2CO_3 (0.78 mmole, 108 mg) and an appropriate alkyl halide (0.78 mmole). The reaction was stirred overnight at room temperature. The reaction was quenched with water and extracted with CH_2Cl_2. The organic phase was evaporated in vacuo and the residue purified by flash chromatography (silica, gradient from 1:99 to 3:97 CH_3OH/CH_2Cl_2) yielding the title compound as a yellow powder in yields ranging from 15 to 55 %. The following compounds were made using this procedure:

- 2-amino-4-morpholino-6-(4-ethoxyphenyl) pteridine (example 103) was obtained from iodoethane and characterised as follows: MS: m/z (%): 727 ($[2M+Na]^+$, 5), 353 ($[M+H]^+$, 100); UV (MeOH, nm): 211, 302, 410;
- 2-amino-4-morpholino-6-(4-benzyloxyphenyl) pteridine (example 104) was obtained from benzyl bromide and characterised as follows: MS: m/z (%): 727 ($[2M+Na]^+$, 5), 353 ($[M+H]^+$, 100); UV (MeOH, nm): 245, 302, 410;
- 2-amino-4-morpholino-6-(4-(phenethyloxy)-phenyl) pteridine (example 105) was obtained from phenylethyl bromide and characterised as follows: MS: m/z (%): 428 ($[M+H]^+$, 100); UV (MeOH, nm): 245, 303, 410;

- 2-amino-4-morpholino-6-(4-phenoxy-butyronitrile) pteridine (example 106) was obtained from 4-bromobutyronitrile and characterised as follows: MS: m/z (%): 391 ($[M+H]^+$, 100);
- 2-amino-4-morpholino-6-(4-propoxy-phenyl) pteridine (example 107) was obtained from propyl iodide and characterised as follows: MS: m/z (%): 367 ($[M+H]^+$, 100);
- 2-amino-4-morpholino-6-(4-phenoxy-butyric acid ethyl ester) pteridine (example 108) was obtained from ethyl-4-bromobutyrate and characterised as follows: MS: m/z (%): 439 ($[M+H]^+$, 100);
- 2-amino-4-morpholino-6-(4-phenoxy-acetic acid ethyl ester) pteridine (example 109) was obtained from ethyl bromoacetate and characterised as follows: MS: m/z (%): 843 ($[2M+H]^+$, 100), 411 ($[M+H]^+$, 100;
- 2-amino-4-morpholino-6-(4-(2-methoxyethoxy)-phenyl) pteridine (example 110) was obtained from 2-bromoethylmethylether and characterised as follows: MS: m/z (%): 787 ($[2M+H]^+$, 25), 383 ($[M+H]^+$, 100); and
- 2-amino-4-morpholino-6-(4-butoxy-phenyl)-pteridine (example 111) was obtained from bromobutane and characterised as follows: MS: m/z (%): 381 ($[M+H]^+$, 100).

Examples 112 to 120 - synthesis of 2-amino-4-alkylamino-6-aryl-pteridines and 2-amino-4-arylalkylamino-6-aryl-pteridines

To a suspension of 2-acetylamino-4-(1,2,4-triazolyl)-6-[3,4-(dimethoxyphenyl)]pteridine (0.5 mmole) in dioxane (5 ml) was added the desired alkylamine or arylalkylamine (1 mmole). The suspension was stirred at room temperature for 16 hours. The solvent was evaporated *in vacuo* yielding crude 2-acetylamino-4-alkylamino-6-[3,4-(dimethoxyphenyl)] pteridine or 2-acetylamino-4-arylalkylamino-6-[3,4-(dimethoxyphenyl)] pteridine. Deprotection of the acetyl group was achieved by dissolving the crude residue in a mixture of CH_3OH / 20% K_2CO_3 in water (1:1). The solution was stirred at room temperature for 16 hours. Evaporation of the solvents *in vacuo*, followed by purification of the residue by preparative TLC (silica, using a CH_3OH/CH_2Cl_2 mixture (5:95) as the eluent) afforded the desired compound as a yellow powder in yields ranging from 30 to 65 %, depending upon the starting alkylamine or arylalkylamine.

The following compounds were synthesized according to this procedure and characterized as follows:

- 2-amino-4-(diethanolamino)-6-[[3,4-(dimethoxyphenyl)]pteridine (example 112) synthesized from diethanolamine; MS: *m/z* (%): 387 ($[M+H]^+$, 100);
- 2-amino-4-(benzylamino)-6-[[3,4-(dimethoxyphenyl)]pteridine (example 113) synthesized from benzylamine; MS: *m/z* (%): 389 ($[M+H]^+$, 100);
- 2-amino-4-(phenylethylamino)-6-[[3,4-(dimethoxyphenyl)]pteridine (example 114) synthesized from phenethylamine; MS: *m/z* (%): 403 ($[M+H]^+$, 100);

- 2-amino-4-(4-methyl-piperidine)-6-[[3,4-(dimethoxyphenyl)]pteridine (example 115) synthesized from 4-methyl-piperidine; MS: *m/z* (%): 381 ($[M+H]^+$, 100);
- 2-amino-4-(2-thienylmethylamino)-6-[[3,4-(dimethoxyphenyl)]pteridine (example 116) synthesized from 2-thienylamine; MS: *m/z* (%): 395 ($[M+H]^+$, 100);
- 2-amino-4-(1,2,3,6-tetrahydropyridino)-6-[[3,4-(dimethoxyphenyl)] pteridine (example 117) synthesized from 1,2,3,6-tetrahydropyridine; MS: *m/z* (%): 365 ($[M+H]^+$, 100);
- 2-amino-4-thiomorpholine-6-[[3,4-(dimethoxyphenyl)]pteridine (example 118) synthesized from thiomorpholine; MS: *m/z* (%): 385 ($[M+H]^+$, 100);
- 2-amino-4-((*R*)-sec-butylamine)-6-[[3,4-(dimethoxyphenyl)]pteridine (example 119) synthesized from (*R*)-sec-butylamine; MS: *m/z* (%): 355 ($[M+H]^+$, 100); and
- 2-amino-4-((*S*)-sec-butylamine)-6-[[3,4-(dimethoxyphenyl)]pteridine (example 120) synthesized from (*S*)-sec-butylamine; MS: *m/z* (%): 355 ($[M+H]^+$, 100).

Example 121 – mixed lymphocyte reaction assay

Pteridine derivatives were first dissolved (10mM) in dimethylsulfoxide (hereinafter referred as DMSO) and further diluted in culture medium before use for the following *in vitro* experiments. The commercially available culture medium consisted of RPMI-1640 + 10% foetal calf serum (FCS). Some pteridine derivatives described in the previous examples (as indicated in table 3 below) were tested in the following mixed lymphocyte reaction (MLR) assay.

Peripheral blood mononuclear cells (hereinafter referred as PBMC) were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (Nycomed, Maorstua, Norway). Allogeneic PBMC or Eppstein-Barr Virus-transformed human B cells [commercially available under the trade name RPMI1788 (ATCC name CCL156)] which strongly express B7-1 and B7-2 antigens were used as stimulator cells after irradiation with 30 Gy. MLR was performed in triplicate wells. After 5 days incubation at 37°C, 1 µCi [^{3}H]-thymidine was added to each cup. After a further 16 hours incubation, cells were harvested and counted in a ß-counter. Inhibition of proliferation by a compound (drug) described in some of the previous examples was counted using the formula:

$$\% \text{ inhibition} = \frac{(\text{cpm} + \text{drugs}) - (\text{cpm cult. med})}{(\text{cpm} - \text{drugs}) - (\text{OD cult. med})} \times 100$$

wherein cpm is the thymidine count per minute. The MLR assay is regarded by those skilled in the art as an *in vitro* analogue of the transplant rejection since it is based on the recognition of allogeneic major histocompatibility antigens on the stimulator leukocytes, by responding lymphocytes.

Table 3 below shows the IC_{50} values for various pteridine derivatives of this invention in the MLR test. The IC_{50} value represents the lowest concentration of said pteridine derivative (expressed in µmole/l) that resulted in a 50 % suppression of the MLR.

TABLE 3

Example n°	MLR	Example n°	MLR	Example n°	MLR
68	0.8	72	8.6	73	6.4
74	1.1	75	4.1	76	2.1
77	2.5	78	6.5	79	7.8
80	4.1	83	3.9	84	4.0
88	4.9	89	0.8	90	10.0
91	10.0	92	2.9	96	4.1
97	3.3	98	3.9	99	4.1
100	0.9	101	0.6	102	4.3
103	3.7	107	6.8	110	5.4
112	6.0	114	6.0	115	0.8
116	4.1	117	0.9	118	0.4
119	0.5	120	0.2		

Example 122 - TNF-α assay

Peripheral blood mononuclear cells (herein referred as PBMC), in response to stimulation by lipopolysaccharide (hereinafter LPS), a gram-negative bacterial endotoxin, produce various chemokines, in particular human TNF-α. Inhibition of the activation of PBMC can therefore be measured by the level of suppression of the production of TNF-α by PBMC in response to stimulation by LPS.

Such inhibition measurement was performed as follows: PBMC were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (commercially available from Nycomed, Norway). LPS was then added to the PMBC suspension in complete medium (10^6 cells /ml) at a final concentration of 1 µg/ml. The pteridine derivative to be tested was added at different concentrations (0.1 µM, 1 µM and 10 µM) and the cells were incubated at 37°C for 72 hours in 5% CO_2. The supernatants were collected, then TNF-α concentrations were measured with an anti-TNF-α antibody in a sandwich ELISA (Duo Set ELISA human TNFα, commercially available from R&D Systems, United Kingdom). The colorimetric reading of the ELISA was measured by a Multiskan RC plate reader (commercially available from ThermoLabsystems, Finland) at 450 nm (reference wavelength: 690 nm). Data analysis was performed with Ascent software 2.6. (also from ThermoLabsystems, Finland): a standard curve

(recombinant human TNFα) was drawn and the amount (pg/ml) of each sample on the standard curve was determined.

The % suppression of human TNFα production by the pteridine derivatives of the invention (drugs) was calculated using the formula :

$$\% \text{ suppression} = \frac{\text{pg/ml in drugs} - \text{pg/ml in cult. med.}}{(\text{pg/ml in cult. med.} + \text{LPS}) - \text{pg/ml cult. med.}}$$

Table 4 below shows the IC_{50} values (expressed in μM) of the tested pteridine derivatives in the TNF-α assay.

TABLE 4

Example n°	TNF-α	Example n°	TNF-α	Example n°	TNF-α
72	8.4	74	2.5	77	4.9
83	4.4	88	4.3	89	0.5
91	7.3	92	5.0	94	5.5
96	7.7	97	10.0	98	2.9
102	2.1	103	10.0	107	10.0
108	0.9	109	0.6	110	7.2
112	3.4	113	6.5	114	7.1
115	0.3	116	7.2	117	0.5
118	0.5	119	0.2	120	0.3